CARIOUS LESIONS IN COTTON RAT MOLARS

III. EFFECT OF THE PARTICLE SIZE AND THE CONSISTENCY OF THE PURIFIED RATION ON THE INCIDENCE AND TYPE OF CARIOUS LESIONS

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SIX FIGURES

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When cotton rats *(Sigmondon hispidus hispidus)* were maintained on a purified diet for 14 weeks after weaning, their molar teeth were found to have been susceptible to the initiation and development of carious lesions (Shaw, Schweigert, McIntire, Elvehjem and Phillips, '44). When the sucrose in this ration was replaced by dextrin or starch, the amount of tooth decay in cotton rats maintained on the resulting rations was much lower than in litter mates fed the sucrose ration (Schweigert, Shaw, Phillips and Elvehjem, '45). The feeding of mineralized whole milk as the sole source of nutrients, or of diets produced by the isocaloric replacement of part of the sucrose by lard, butterfat or casein, resulted in a decreased initiation and development of carious lesions (Schweigert, Shaw, Zepplin and Elvehjem, '46).

The effect of mastication on dental caries in the cotton rat was studied by extraction of the molars in one upper quadrant prior to the beginning of the experiment (Shaw, '47). The results indicated that there was a much less important effect of mastication of the purified diet upon the

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dental caries attack rate than had been observed previously in the white rat fed the coarse, Hoppert-Webber-Canniff diet (Sognnaes, '41; Ginn, '42). However, cotton rats fed either the purified diet or a ration composed of whole milk solids in liquid form had substantially lower average numbers and average extents of carious lesions than littermates fed the same rations in dry form (Anderson, Smith, Elvehjem and Phillips, '48).

Mechanical lesions are not known to precede the initiation of tooth decay in human teeth. Therefore, for any animal to be used for fundamental caries research it is of considerable importance to explore the relationships of diets with a wide variety of physical properties to the maintenance of teeth. The present series of experiments was conducted with cotton rats to study: (1) the morphology of the carious lesions in relation to tooth structure and masticatory stresses; and (2) the effects of diets of varying particle size and varying consistency on the production of tooth decay.

EXPERIMENTAL

The cotton rats used in these experiments were the offspring of animals which had been selected for breeding purposes through several generations because of their high and predictable susceptibility to tooth decay. Stock colony cotton rats were fed the ration composed of natural foodstuffs described previously (Shaw, '47). From weaning until the termination of an experiment, control and experimental rats were housed in individual cages with floors made of coarse wire screen.

Ration 100, a slight modification of the caries-producing diet of Shaw, Schweigert, McIntire, Elvehjem and Phillips ('44), was used as the basal ration. The composition of ration 100 and its various isocaloric modifications are presented in table 1. When these rations were offered ad libitum, with ample drinking water, a high percentage of the rats grew well and seemed to be normal in activity and appearance for the duration of the experimental periods. Some

TABLE 1

Composition of ration in grams of each constituent

CONSTITUENTS	RATION														
	100	120	130	140	104	134	144	100A	100B	120B	130A	130B	140A	101	102
Sucrose	67	58	49	40	..	..	..	67	67	58	49	49	40	..	..
Powdered sugar [1]	..	..	..	..	67	49	40	..	..	..	..	..	..	..	..
Dextrin — fine	..	..	..	..	..	..	..	..	..	..	..	..	..	67	..
Dextrin — coarse	..	..	..	..	..	..	..	..	..	..	..	..	..	..	67
Casein [2]	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Corn oil [3]	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Lard	..	4	8	12	..	8	12	..	..	4	8	8	12	..	..
Salt mixture [4]	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
1:20 liver concentrate	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Whole liver substance	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Cellu flour	..	..	..	..	..	..	..	10	20	20	10	20	10	..	..
Fat content as per cent by weight of digestible components	4.8	9.1	13.8	19.1	4.8	13.8	19.1	4.8	4.8	9.1	13.8	13.8	19.1	4.8	4.8
Carbohydrate content as per cent by weight of digestible components	64.4	58.6	52.1	44.9	64.4	52.1	44.9	64.4	64.4	58.6	52.1	52.1	44.9	64.4	64.4

[1] Three per cent corn starch added to prevent caking.

[2] "De-vitaminized" casein, Sheffield Farms, New York. Each 24 gm had had incorporated in them 350 μg of thiamine hydrochloride, 350 μg of riboflavin, 350 μg of pyridoxine hydrochloride, 2.0 mg of calcium pantothenate, 2.5 mg of nicotinic acid, 100 mg of choline chloride, 100 mg of inositol, and 30 mg of para-aminobenzoic acid.

[3] Mazola — Each 5 gm had had incorporated into them 1.1 mg of beta-carotene, 300 I.U. of irradiated ergosterol, 0.6 mg of 2-methyl-1,4-naphthoquinone, and 5.0 mg of alpha-tocopherol.

[4] J. Dent. Res., *26:* 47, 1947.

deaths occurred, due usually to intercurrent infections, occasionally to the formation of hair balls in the stomach, but independent of the composition of the ration.

In the first experiment cotton rats were maintained on ration 100 for varying periods of from 6 to 14 weeks to provide histologic material. Litter mates were sacrificed at successive intervals by ether anesthesia, decapitated and the heads fixed in a 10% formalin solution for 48 hours. An upper and a lower quadrant were used to evaluate the number and extent of carious lesions by the grinding and observation procedure (Shaw, Schweigert, McIntire, Elvehjem and Phillips, '44). The other quadrants were dehydrated and imbedded in methyl methacrylate (Sognnaes, '47). A sagittal anteroposterior ground section was made of each jaw for detailed study of the carious lesions.

In the second experiment various isocaloric modifications of ration 100 were made to alter the particle size of its components and to alter the texture and the consistency of the resulting rations. The approximate energy value of that aliquot of each ration listed in table 1 was 409 cal., as calculated with the Atwater standards of 4, 9 and 4 cal. per gram of carbohydrate, fat and protein, respectively. No accurate energy value could be assigned for the 4 gm of liver concentrates in the above aliquot of each ration.

In the first series of rations (120, 130 and 140) progressive increases in the fat content were made by the isocaloric substitution of 4, 8 or 12 gm of lard for 9, 18 or 27 gm, respectively, of each 67 gm of sucrose in the basal ration. These levels of substitution were chosen so that two would be lower and the third slightly higher than the lower level at which a reduction in tooth decay was observed by Schweigert et al. ('46). In this way a closer estimate could be made of the lowest level of fat and the highest level of sucrose at which a substantial reduction in the average number and extent of carious lesions per cotton rat could be produced. Marked differences in the consistency of the ration resulted with isocaloric substitution. A progressive decrease in the

granular, particulate texture which was characteristic of ration 100 and a progressive increase in the greasiness and plasticity of the rations was observed with each increase in lard content and decrease in sucrose content, until ration 140 had the consistency of a somewhat granular, malleable dough.

The particle size and consistency of rations 100, 130 and 140 were altered by replacement of the granulated sucrose, the only coarse ingredient, by finely powdered sugar in order to give rations 104, 134 and 144. Ration 104 was extremely fine and almost dusty in nature, and its particles did not adhere to each other. The particles in rations 134 and 144 had an increasing tendency to adhere to each other, but did not feel as greasy and were not as pasty or malleable as their counterparts (130 and 140) prepared with granulated sucrose. On the basis of appearance and touch, ration 144 could be described as being roughly comparable in consistency to ration 120 which contained much less fat.

The consistency of rations 100, 120, 130 and 140 was also altered by the incorporation in the rations of cellu flour, which has been shown independently to have no effect on the initiation and development of carious lesions in the cotton rat (Howell, Schlack, Taylor and Berzinskas, '48). The presence of cellu flour materially decreased the greasy texture of the rations and maintained them in a more particulate form due to the greater dispersion of the fat, but did not alter the relative distribution of calories from fat and carbohydrate nor the relation of calories to minerals and vitamins.

In another series of rations the sucrose was entirely replaced by either fine or coarse dextrin (ration 101 and 102, respectively). The fine dextrin had particles which were sufficiently small to pass readily through a 60-mesh sieve. The coarse dextrin was composed of particles of varying size: 30% were retained on a 20-mesh sieve and an additional 50% were retained on a 60-mesh sieve, while the remainder passed through both. Ration 101 had a consistency and

powdery nature similar to those of ration 104, where sucrose had been replaced by powdered sugar. Ration 102 was much coarser than ration 100 and was outstandingly different from other rations studied because of the extensive mastication necessitated in the consumption of the coarse and flinty dextrin particles.

The litters of cotton rats used in the second experiment were distributed as equally as possible among the control and experimental groups. All animals were continued on the dietary regimen for 14 weeks, when they were sacrificed and the heads fixed in 95% ethanol. The number of carious lesions and the amount of tooth substance involved in each lesion were evaluated by the grinding and observation procedure of Shaw, Schweigert, McIntire, Elvehjem and Phillips ('44).

RESULTS

A series of photomicrographs of ground sections is presented in figures 1 to 6, inclusive, to indicate certain types and stages of the carious lesions which develop in the molars of cotton rats. A description of the anatomical characteristics of the molar teeth and of the readily visible carious lesions accompanies the illustrations.

Accumulation of food debris

The sulci (grooves) usually contain some debris, which differs widely in amount, color, histologic appearance and readiness with which the particles can be removed with a fine probe. The presence of such material does not appear to be associated necessarily with fracture of the cusps nor with an early destruction by carious lesions of adjacent tooth structures. Frequently sulci with extensive tooth decay contain little foreign matter, as in figures 2 and 5; certain sulci may be full of material and yet be accompanied by little or no evidence of tooth decay, as in figure 1. When the grooves are as narrow and deep as those in the cotton rat, one would

scarcely expect any appreciable interchange of the materials in the sulci, especially in the case of fibrous, or tightly wedged, particles. Since some particles removed from the sulci during the examination for carious lesions resembled the stock ration, or fragments of wood shavings, to neither of which the rats had had access since they were weaned, some particles probably were forced into the sulci prior to weaning.

The accumulation of material was rare on the surfaces of the molar teeth which face the cheek, the tongue or the adjacent teeth. At times a light brown, hard, "candied" residue was observed on the third of the molar crowns nearest the gingiva, and was especially noticeable on first molars. This accumulation was not associated with carious lesions of these surfaces and was not sufficiently extensive to cause any detectable damage to the supporting structures of the teeth during the length of the experimental period. Soft, pasty residues were observed only on the smooth surfaces of the molars in cotton rats from which the major salivary glands had been removed, and even then did not appear to be associated with the initiation of lesions (Shaw and Weisberger, '49).

Position of carious lesions

The most common place where carious lesions occurred in the molar teeth was at the bases and on the anterior and posterior surfaces of the sulci (figs. 2 and 3); the next most common site was on the surfaces (figs. 2 and 6) in contact with the adjacent teeth. Usually the point of origin of the lesions was a considerable distance below the occlusal surface. The earliest lesion detected consisted of an area in the enamel which was soft and pigmented. The region involved in these early lesions differed greatly in size, varying from a small area of penetration to rather wide, diffuse areas of invasion. These lesions occurred prior to any fracture or detectable mechanical injury of the enamel. Lesions on the tooth surfaces which face the cheek and tongue were observed

with extreme rarity. As yet, no subgingival lesions have been detected. The exposed dentin on the occlusal surface of the molars was not readily susceptible to tooth decay. The latter type of lesion did occur frequently in third molars in the period immediately following their eruption into the oral cavity and is particularly extensive in cotton rats, where the process of eruption of the third molars is unusually late (fig. 1).

The rate of progress of carious lesions was found to be very rapid. Well-developed lesions of the enamel with slight dentin penetration were present in highly susceptible animals as early as the 5th week on experiment. Almost complete destruction of the molar crowns in all quadrants was observed in some susceptible animals by the 10th week.

Fracture of cusps did not occur prior to initial lesions in the molars of cotton rats maintained on ration 100. When large areas of molar cusps had been undermined by the carious process the forces of mastication were sufficient to produce secondary fracture with loss of fragments of enamel and dentin.

Alterations in particle size and consistency of rations

The effects of the various purified rations upon the average number and average extent of carious lesions during the 14-week experimental period are presented in table 2. Ration 120, in which 9 of each 67 gm of sucrose in ration 100 had been replaced by 4 gm of lard, did not result in any appreciable difference in the average number and average extent of carious lesions from those values observed for the litter mates fed ration 100. The feeding of ration 130, in which an additional 9 gm of sucrose had been replaced by 4 gm of lard, resulted in a slightly lower average number and average extent of carious lesions than were found in the control litter mates. When ration 140 was fed, where a total of 27 gm of each 67 gm of sucrose in ration 100 had been isocalorically replaced by lard, there was a strikingly lower average number and average extent of carious lesions than in the

TABLE 2

The average number and extent of carious lesions observed in cotton rats maintained for 14 weeks on various purified rations

RATION NO.	NUMBER OF COTTON RATS	AVERAGE NUMBER OF CARIOUS LESIONS	S.E.M.[1]	C.R.[2]	AVERAGE EXTENT OF CARIOUS LESIONS	S.E.M.[1]	C.R.[2]
100	14	24.2	1.8	> 0.3	66 +	8 +	> 0
120	12	24.9	1.4		66 +	8 +	
100	29	27.3	1.1	> 2.6	79 +	5 +	> 3.7
130	27	23.4	1.0		55 +	4 +	
100	15	24.8	1.2	> 4.9	65 +	7 +	> 4.9
140	15	15.9	1.3		27 +	4 +	
100	11	27.0	1.5	> 0.7	78 +	7 +	> 0.9
104	8	28.9	2.1		88 +	9 +	
130	9	25.0	0.7	> 0.4	66 +	4 +	> 1.5
134	7	24.1	2.3		52 +	8 +	
140	8	15.0	1.3	> 0.5	22 +	4 +	> 0.4
144	9	16.0	1.5		23 +	6 +	
100	6	26.5	1.2	\ 0.7	85 +	8 +	\ 0.3
100A	8	27.9	1.7	×	88 +	7 +	×
100B	7	26.0	1.5	/ 0.3	89 +	9 +	/ 0.3
120	9	23.7	1.4	> 0.8	59 +	10 +	> 0.4
120B	7	21.9	2.0		53 +	11 +	
130	9	20.8	1.9	> 0.3	40 +	8 +	> 0.4
130A	7	22.0	2.8		46 +	11 +	
130	8	23.8	1.0	> 0.2	55 +	4 +	> 0.6
130B	8	23.4	1.8		51 +	6 +	
140	8	15.3	1.9	> 0.7	23 +	3 +	> 1.1
140A	9	17.1	1.7		29 +	4 +	
100	9	25.9	1.1	— 9.8	81 +	4 +	— 13.2
101	8	10.4	1.2	× 15.6	23 +	2 +	× 16.4
102	8	4.4	0.9	— 4.0	9 +	2 +	— 6.2

[1] Standard error of mean.

[2] Critical ratio.

litter-mate controls. On the basis of experimental data, the critical level of fat at which occurred a substantial reduction in the average number and average extent of carious lesions appeared to lie between 13.8 and 19.1%, with no appreciable protective effect at a level of 9.1%. Or, to express the relationship in terms of sucrose, no substantial reduction in the average number and average extent of carious lesions occurred until the sucrose level was reduced to 52.1% or less by isocaloric replacement with fat.

The substitution of finely powdered sugar for the granulated sucrose in rations 100, 130 and 140 did not alter the initiation and development of carious lesions in the cotton rat. Similarly, the addition of indigestible material in the form of cellu flour to rations 100, 120, 130 and 140 did not affect the initiation and development of carious lesions.

The complete replacement of the granulated sucrose in ration 100 by fine or coarse dextrin resulted in striking reductions in the average number and average extent of carious lesions, as reported previously (Shaw, Schweigert, McIntire, Elvehjem and Phillips, '44). The ingestion of ration 102 containing coarse dextrin resulted in an extensive degree of attrition and a widespread initial fracturing of the molar cusps, with a resulting modification of the normal structural characteristics of the grooves. Those few carious lesions in the cotton rats fed the coarse dextrin diet were most frequently found on the dentin surfaces exposed by these fractures and not on the few remaining intact enamel surfaces. The effect of the replacement of granulated sugar by coarse dextrin appeared to entail not solely a replacement of the form of carbohydrate but, in addition, a modification of the structure of the teeth and, possibly in this way, a modification of caries susceptibility.

No more attrition or fractures occurred in the molars of cotton rats fed the fine dextrin diet 101 than in those of their litter mates fed ration 100. The carious lesions produced in cotton rats fed diet 101 were characteristic of early lesions in control animals and appeared to progress ex-

tremely slowly. Since cotton rats disliked the finely powdered dextrin diet sufficiently that they refused or spilled a high percentage of the amount offered, an insufficient quantity was eaten to allow normal growth and well-being. Undoubtedly this has been a complicating factor in the initiation and development of carious lesions on this dietary regimen. A comparably drastic caloric restriction has been found to be associated with a greatly reduced incidence and extent of carious lesions in the cotton rat (Shaw, '48). Thus the feeding of diets where the sucrose had been replaced by either coarse or fine dextrin resulted in complications which could not be attributed to the replacement of sucrose per se.

Rates of growth

The average rates of growth during the experimental periods and the average final body weights for the various groups of cotton rats in these investigations were normal, with one exception. The cotton rats fed the fine dextrin diet grew at a much slower rate and attained much lower final body weights than the litter mates fed ration 100. Rations 104, 134 and 144, in which the sucrose had been replaced by powdered sugar, allowed the same rates of growth and final body weights as their counterparts, 100, 130 and 140. The fine particle size of the powdered sugar materially affected the over-all particle size and consistency of the rations but, except for the occasional animal fed ration 104, the rations containing powdered sugar were not disliked by the cotton rats to such an extent that reduced amounts were eaten. Despite the comparable particle size of the components of rations 104 and the fine dextrin ration, 90% of the cotton rats offered the former ration ate it and did well, while all the animals offered the fine dextrin ration ate little and grew poorly. Part of the difference in the acceptance of the two rations seemed to be due to the ability of the corn oil to cause the particles of powdered sugar to adhere loosely, whereas the same amount of corn oil was insufficient to cause any binding of the fine dextrin particles. The addition of

various amounts of cellu flour to the diets did not consistently alter the rates of growth of the cotton rats in these experiments. Similarly, supplements of cellu flour did not increase the survival rate of cotton rats fed purified rations, as has been suggested previously by Howell, Schlack, Taylor and Berzinskas ('48). Survival in the above experiment seemed to be dependent to a great extent upon the unpredictable presence or absence of intercurrent infections in the animal quarters.

DISCUSSION

Histologic studies of the molar teeth from cotton rats which had been maintained on purified ration 100 failed to indicate the presence of any mechanical injury of the caries-susceptible enamel surfaces previous to the initiation of carious lesions. Since the grooves in cotton rat molars are extremely narrow, and since most early carious lesions were initiated toward the bases of the sulci or on the surfaces adjacent to other teeth, the caries-susceptible areas would appear to be well protected from physical trauma. The replacement of sucrose, the only coarse constituent in rations 100, 130 and 140, which require little mastication, by finely powdered sugar resulted in rations with very finely subdivided particles requiring no appreciable amount of mastication. The fact that this reduction in particle size did not alter the initiation nor the rate of development of carious lesions further indicated that these conditions are not dependent upon preliminary injury to the enamel surfaces.

Tests on the replacement of the soluble carbohydrate, sucrose, by a less soluble one, dextrin, were inconclusive because of certain uncontrolled variants: partial caloric restriction in the case of replacement by fine dextrin, and extensive abrasion and fracture in replacement by coarse dextrin. One point demonstrated by these studies was that fracture of the tooth surfaces in the cotton rat is not necessarily associated with a high degree of initiation and development of carious lesions; extensive fractures could be associated with a lower incidence of dental caries than when

the same diet was fed in a form not conducive to mechanical injury of the teeth. In this regard there is a clear-cut difference between the early experiments with the common laboratory rat using the coarse particle, Hoppert-Webber-Canniff diet and investigations in the cotton rat using purified rations.

The mechanism by which isocaloric replacement of dietary carbohydrate caused reductions in the initiation and development of carious lesions needs considerable further investigation. Once the ratio of fat to sucrose in a ration had been increased until a slight reduction in the incidence and extent of carious lesions occurred, a small further increase in this ratio resulted in a further rather considerable reduction in the dental caries experience. The ration (140) with the highest fat:sucrose ratio contained 44.9% by weight of the digestible components as sucrose and had a fat content of only 19.1%. Yet cotton rats fed this ration had a 36% lower average number of carious lesions and a 59% lower average extent of carious lesions than the control litter mates. The various fat: sucrose ratios tested had the same effect upon the initiation and development of carious lesions regardless of whether the carbohydrate in the ration was coarse granulated sucrose or fine powdered sugar or whether large amounts of cellu flour had been added. By both procedures, rations with increasingly higher fat: sucrose ratios could be produced in more particulate, somewhat non-greasy forms instead of greasy masses through the distribution of the fat over a greatly increased number of particles, both with and without a dilution of the nutrients by an inert, non-nutritious material. The lack of a demonstrable relationship between the consistency of the ration, the distribution of the fat over a large or small number of particles, and the initiation and development of carious lesions in the cotton rat strongly suggests that the increasing fat content of the diet was not effective in reducing the dental caries attack rate through a mechanical alteration in the form of the diet or its constituents.

SUMMARY

1. Carious lesions in cotton rats maintained on purified rations were most common deep in the sulci and on the proximal surfaces of the molar teeth. Occasional lesions were found on exposed dentin of the occlusal surfaces, but almost none on surfaces which face the cheek or tongue.

2. Histologic examination of ground sections of cotton rat jaws indicated that the carious lesions in the sulci and on the proximal surfaces began on intact enamel surfaces well below the occlusal plane. There was no evidence of fracture previous to the initiation of carious lesions except in those cotton rats fed a ration in which sucrose had been replaced by coarse dextrin.

3. The isocaloric replacement with lard of 9 gm of each 67 gm of sucrose in the basal ration did not alter the number nor the extent of carious lesions in the cotton rats. The isocaloric replacement by lard of 18 gm or 27 gm of each 67 gm of sucrose in the basal ration resulted in increasingly significant reductions in the dental caries attack rate.

4. The complete replacement of the sucrose in rations with varying fat content by finely powdered sugar altered the particle size of the rations greatly but did not result in any alteration in the average number nor in the average extent of carious lesions.

5. The addition of large amounts of crude indigestible fiber in the form of cellu flour to rations with varying fat content greatly altered the consistency of the rations but did not alter the average number nor the average extent of the carious lesions.

6. Replacement of sucrose in the control ration by fine or coarse dextrin resulted in highly significant reductions in the dental caries experience. These reductions could not be attributed entirely to the simple replacement of a soluble carbohydrate by a less soluble or insoluble one.

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PLATE 1

EXPLANATION OF FIGURES

Figs. 1–6 Ground sections of cotton rat jaws in the vertical antero-posterior plane, imbedded in polymerized methyl methacrylate. The large number in the lower right corner of each photograph is the figure designation. The small characters on the upper margin of each photograph are from the scoring code to designate the sulci immediately below them, and will be referred to in the description of each figure in order to locate the lesions. The letter A is placed on each photograph to designate the anterior aspect. In all cases the occlusal plane is at the top of the photograph.

Fig. 1 A lower left jaw of a cotton rat which had been maintained on the basal purified ration for 8 weeks. The narrow and extremely deep sulci, the rather thin border of enamel, and the packing of debris in the sulci can be seen. Examination of the sulci in the first and second molars discloses only small enamel lesions which are not readily visible at this magnification. In sulci 37 and 39 of the third molar extensive lesions can be seen. The lesion in sulcus 37 appears to have begun on the exposed dentin of the occlusal surface. The lesion in sulcus 39 may have begun on the posterior enamel surface or on the dentin of the occlusal surface. In both cases there has been a considerable loss of the tooth structures above the lesion. × 15.

Fig. 2 A lower right jaw of a cotton rat which had been maintained on the basal purified ration for 14 weeks. The carious lesions in this jaw are more frequent and more extensive than those in figure 1. In sulcus 24 of the first molar there is a lesion which appears to have begun on the posterior surface and progressed until the portion of the cusp undermined by the lesion has been fractured under the pressure of mastication. An extensive lesion has occurred in sulcus 28, with a penetration of the posterior surface until fracture has occurred. On the anterior side of sulcus 28 there is a small pigmented area in the enamel indicative of an early lesion. On the posterior surface of the first molar there is a small but rather rapidly progressing enamel lesion which has not reached the dentin. In the second molar there has been a lesion on the anterior surface or in sulcus 32 or in both. The exact origin cannot be determined since fracture of the cusp has occurred, obliterating the original cusp and the sulcus. In sulcus 34 there is a large lesion on the posterior surface which has penetrated into the dentin but which has not yet resulted in any loss of tooth structure due to this undermining. In sulcus 40 of the third molar there is an area of involved enamel on both the anterior and posterior surfaces directly opposite each other. The carious area on the posterior surface has penetrated into the dentin, while the lesion on the proximal surface has not progressed completely through the enamel. This jaw illustrates the customary occurrence of all stages of lesions in one jaw, all of which can be evaluated with reasonable accuracy in so far as their origin is concerned except the lesion involving both sulcus 32 and the anterior surface of the second molar. × 15.

Fig. 3 A portion of a lower left third molar. On the posterior surface of sulcus 39 near the occlusal surface is a small lesion with a loss of enamel structure and dentin penetration. Somewhat deeper in the sulcus are two small pigmented enamel lesions opposite each other on the anterior and posterior surfaces. Toward the bottom of the sulcus there is an area of penetration in the dentin which appears to have been associated with some lesion in which the enamel area involved is not represented in the plane of this section. × 60.

Fig. 4 A lower right third molar and the posterior surface of the second molar. On the anterior surface of the third molar a wide, pigmented enamel lesion is visible. The posterior surface of sulcus 40 has a carious area with a wide enamel and a wide and deep dentin involvement which has not been complicated by fracture in mastication. A wide area of enamel and a deep area of dentin have been involved in the carious process. × 45.

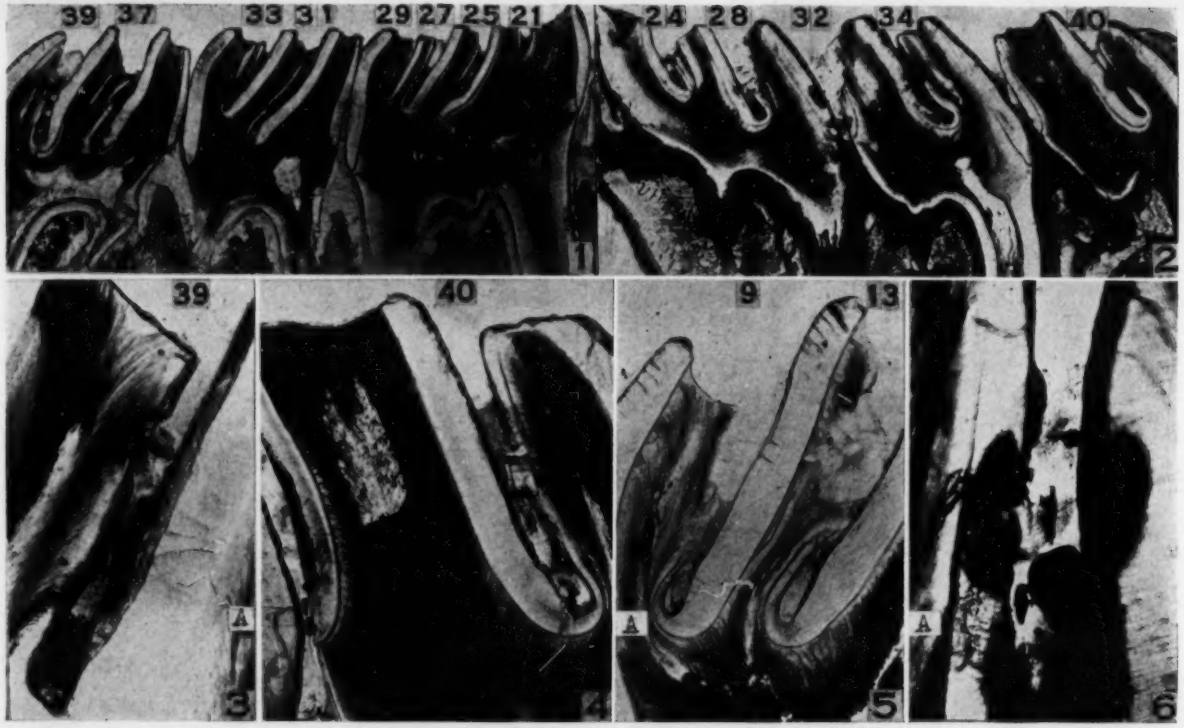

Fig. 5 An upper left second molar. In sulci 9 and 13 are extensive lesions on the anterior surface in which fracturing of the cusps has also occurred, but after the initiation of the lesions. The intact dentin at the occlusal surface of each sulcus would seem to indicate that these lesions did not begin on the dentin surface. The irregular areas on the posterior surface of the second molar and the proximal surface of the third molar are artefacts due to the inclusion of air bubbles when the block containing this jaw was being mounted on the slide. × 45.

Fig. 6 The proximal surfaces of lower right first and second molars. A carious lesion is shown on both the posterior surface of the first molar and on the anterior surface of the second molar. In the former lesion there has been a definite penetration of the dentin. × 60.

DETERMINATION OF SOME ESSENTIAL AMINO ACIDS IN SEVERAL UNCOOKED AND COOKED MEXICAN FOODSTUFFS

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While in other countries meat, eggs, and milk are the principal sources of protein in the diet, in Mexico they are not commonly used, because of their excessive cost or because of traditions very firmly established among the people. The usual Mexican diet among the poorer classes consists chiefly of corn, beans and pulque, supplemented with some vegetables, among which chiles and, in the city of Mexico, rice are outstanding.

The present study has as its purpose to add to our knowledge the nutritive value of these foodstuffs. Data concerning their caloric, vitamin, and mineral content may be found in the articles of Cravioto et al. ('45b) and Cravioto and Miranda ('47) but little is known of the quantities they contain of the so-called essential amino acids. Among the first papers of this kind relating to Mexican foods is that of Giral and Cravioto ('41) on the threonine content of corn, but there is no information available concerning food products prepared for consumption. For this reason the present study also considers the composition of tortillas, cooked beans, and cooked rice. It is the belief of the authors that the basic principle for judging whether a source of

proteins is suitable or not is knowledge of the quality of these proteins; that is to say, whether the necessary quantities of essential amino acids are present, since according to some authors (Elvehjem, '46; Krehl et al., '46) a diet rich in poor-quality protein can do more damage than a diet with less protein but with a balanced content of amino acids.

EXPERIMENTAL PROCEDURE

Samples

A sample was taken of the white corn (Zea mays L.) from which tortillas are made following the method commonly used in Mexico as reported by Cravioto et al. ('45a), which is treatment with $Ca(OH)_2$ and heating. Starch was removed from these samples by salivary digestion according to the method of Doty ('41). For the beans, the variety used was one of the most common in Mexico, palacio beans (Phaseolus vulgaris L.), a part of which were cooked with water by boiling three to 4 hours at atmospheric pressure to obtain what is called in Mexico "frijoles de olla" (stewed beans). As with the corn, the starch was removed from both the raw and cooked beans by a modification of the method of Doty. The rice used was the unpolished white product such as is consumed in Mexico, a part of which was cooked using the technique for the preparation of "arroz blanco," as follows: (a) the rice is washed with hot water which is discarded and then with cold water which is discarded too; (b) the rice is drained and heated with oil or lard for 5 to 10 min., the excess of oil being removed; (c) the rice is covered with water and cooked for half an hour, adding salt to taste and water as necessary. For analysis the samples of raw and cooked rice were treated with ether in the Soxhlet extractor for 16 hours to remove the fat. All the grains were finely ground with mortar and pestle and dried *in vacuo* over P_2O_5. A sample of pulque "de tinacal" (factory pulque) was taken and the analysis begun without any special previous treatment.

Nitrogen analysis

For the corn, tortillas, raw beans and cooked beans, the micro-Kjeldahl method was used on duplicate samples of 10 to 20 mg dried over P_2O_5. For the raw and cooked rice the method of Kjeldahl was used on samples of 0.5 gm dried as in the previous case. The pulque was analyzed by the Kjeldahl method, using samples of 25 ml.

Preparation of samples for analysis

Hydrolysates were made on duplicate samples of 100 mg of corn, tortillas, and raw and cooked beans, and of 1.0 gm of raw and cooked rice, each with 10 ml of 10% HCl, by autoclaving 10 hours at 15 lb. for the determination of all amino acids except tryptophan. For this last determination hydrolysis was carried out at 15 lb. for 10 hours with 5 ml of 5 N NaOH on samples of 50 mg for corn, tortillas and raw and cooked beans and of 0.5 gm for raw and cooked rice, the last two with 10 ml of 5 N NaOH. For the analysis of pulque, 500 ml were hydrolyzed with HCl added to give a final concentration of 10%. To make the alkaline hydrolysate, 20 gm of NaOH were added to 100 ml of pulque, giving a concentration of approximately 20%. Both samples were hydrolyzed at 15 lb. for 10 hours. The samples were all neutralized and the pH adjusted to 6.8 after elimination of the humin. They were diluted as desired.

Amino acid analysis

The microbiological method of Stokes et al. ('45) was employed for the determination of amino acids, utilizing *Streptococcus faecalis* and *Lactobacillus delbrückii* LD5. In some cases (beans, cooked beans, rice, cooked rice, and pulque) 2.5 ml of the basal medium were used instead of 5 ml, with the same dilution of the inoculum. The standard solutions were made at the same concentration as in the original method; the final volume in the tubes was 5 ml. The acid produced during incubation was titrated with 0.025

TABLE 1

Amino acid content of the foods analyzed

SUBSTANCE	NITROGEN PER CENT OF DRY-WEIGHT	PER CENT, CALCULATED TO 16% NITROGEN, ON DRY BASIS								
		Lysine	Trypto-phan	Histi-dine	Phenyl-alanine	Leu-cine	Threo-nine	Methio-nine	Valine	Arginine
Corn, defatted [1]	6.91	2.5	0.6	2.3	4.4	19.6	4.7	1.9	5.4	4.4
Tortilla, defatted [1]	6.06	2.2	0.4	1.7	4.3	18.9	3.5	1.7	5.4	3.7
Beans, defatted [1]	6.69	6.0	1.0	2.5	5.4	13.2	5.0	0.7	5.3	4.7
Beans (cooked), defatted [1]	6.83	5.7	1.0	2.4	5.3	11.8	4.8	0.7	5.1	4.7
		PER CENT, CALCULATED TO 16.8% NITROGEN, ON DRY BASIS								
Rice, defatted	1.32	3.3	1.5	2.1	4.6	9.7	4.5	1.9	6.7	6.3
Rice (cooked), defatted	1.17	3.3	1.7	1.8	4.8	9.5	4.1	2.0	6.8	5.8
		PER CENT, CALCULATED TO 16% NITROGEN, ON DRY BASIS								
Whole egg proteins [2]		7.2	1.5	2.1	6.3	9.2	4.9	4.1	7.3	6.4

[1] The starch was removed by salivary digestion.

[2] Data from Mitchell and Block ('46).

N NaOH except in the determinations of phenylalanine, in which case 0.05 N NaOH was used. Recovery tests were carried out by adding known amounts of amino acids to samples of corn and tortilla prior to hydrolysis, using 2.5 ml of basal medium per tube.

RESULTS

The amino acid content of the foods analyzed, with the exception of the pulque, is reported in per cent of total protein as referred to dry matter. As recommended by the United States Department of Agriculture ('31), the factor 6.25 was used to calculate the protein content of the corn, tortillas and beans (cooked and uncooked), and the factor,

TABLE 2

Amino acid content of the pulque

NITROGEN GM PER 100 ML	AMINO ACID CONTENT, IN MG PER 100 ML								
	Lysine	Trypto-phan	Histi-dine	Phenyl-alanine	Leu-cine	Threo-nine	Methio-nine	Valine	Arginine
0.14	16.2	2.7	4.7	11.2	10.5	6.4	0.7	6.6	10.9

5.95 for the raw and cooked rice. The results are reported in table 1 with the exception of those relating to the pulque, which appear in table 2, where the amino acids are expressed as milligrams per 100 ml. We wish to point out that in the case of rice cooking causes an appreciable loss of nitrogen, probably because the wash waters which were discarded removed some nitrogen compounds. In terms of dry matter, uncooked rice and the same rice cooked contained 1.32% and 1.17% of nitrogen, respectively.

In table 3 there is a comparison of the data that we have obtained for whole corn and rice, on the basis of 16% of nitrogen. We have not found any reports on the amino acid composition of the bean studied (Phaseolus vulgaris). There are only references to the α and β globulins in the black beans of the Mayas, in which Jones et al. ('38) found some amino acids.

TABLE 3

Comparison of some values reported in the present paper (whole corn and rice) with others observed by different authors

Per cent, calculated to 16% nitrogen, on dry basis

AMINO ACID	WHOLE CORN				RICE			
	Values in the present work	Values from literature			Values in the present work	Values from literature		
		Block and Bolling [1]	Horn et al.[2]	Schweigert [3]		Block and Bolling [1]	Horn et al.[2]	Block [4]
Lysine	2.5	2.5	2.3	2.3, 2.3	3.1	3.2	2.8	3.2
Tryptophan	0.6	0.6			1.4	1.3		1.3
Histidine	2.3	2.4	1.9	2.4, 2.2	2.0	1.5	2.0	1.5
Phenylalanine	4.4	4.5	5.2	4.3	4.4	6.2	4.8	6.7
Leucine	19.6	21.5 ± 2.4		9.6	9.2	7.7		9.0
Threonine	4.7	3.6	3.9	3.1, 3.9	4.3	3.9	3.5	4.1
Methionine	1.9		1.4		1.8	3.4	1.4	3.4
Valine	5.4	4.6 ± 0.7	5.3	5.2	6.3	6.4	6.3	6.3
Arginine	4.4	4.0	4.7	4.7	6.0	7.2	8.2	7.2

[1] Block and Bolling ('45).

[2] Horn et al. ('46, '47a, '47b, '47c, '48a, '48b, '48c).

[3] Schweigert ('48).

[4] Block ('45).

Recoveries of known amounts of amino acids added to substances prior to hydrolysis are quantitative within ± 5%. The results are reported in table 4.

Generally speaking, our data on the amino acid content of whole corn and rice agree with those reported by several investigators. Our data on threonine and methionine in whole corn and on arginine, methionine and phenylalanine in rice are not in accord with those of some authors. We believe that the disagreements are due to some extent to the different methods employed for the analysis of the amino

TABLE 4

Recovery of amino acids added to proteins prior to hydrolysis
(Milligrams per gram of dried material)

AMINO ACID	SAMPLE	CONTENT	ADDED	TOTAL	FOUND	PER CENT RECOVERY
Threonine	Tortilla	12.7	15.0	27.7	26.5	96
Valine	Tortilla	19.9	20.0	39.9	40.7	102
Phenyl-alanine	Tortilla	16.0	15.0	31.0	30.0	97
Leucine	Tortilla	71.9	75.0	146.9	145.5	99
Methionine	Tortilla	6.6	5.0	11.6	11.8	102
Arginine	Tortilla	14.0	15.0	29.0	30.2	104
	Corn	19.7	15.0	34.7	36.8	105
Histidine	Tortilla	6.5	5.0	11.5	11.3	98
	Corn	10.0	5.0	15.0	14.3	95
Lysine	Corn	10.4	10.0	20.4	19.4	95

acids. On the other hand, possibly they may be due to the differences that Doty et al. ('46) observed in the amino acid content of corn because of the influences of genetic factors, and also to the fact, observed by Kik ('41), that the use of fertilizers affects the amino acid content of rice.

DISCUSSION

If we accept the criterion of Mitchell and Block ('46), it is important to evaluate the quality of a protein with respect to its amino acid content by comparing it with whole egg. Experiments, based on growth in the rat, carried out by

these authors show whole egg protein to be perfectly balanced in amino acids. For this reason, at the bottom of table 1 are shown the figures reported by Mitchell and Block for whole egg. In this manner we can calculate the biological value of the foods analyzed in spite of the lack of data concerning isoleucine, tyrosine, and cystine, the last two being considered semi-essential by Block and Bolling ('44). Practically the same criterion has been upheld by Giral ('43), who compared the quantity of amino acids furnished by various foods with that furnished by eating two eggs daily, which he considers to contain sufficient essential amino acids.

According to the point of view of Mitchell and Block, then, it can be stated that corn is more deficient in lysine than in tryptophan, and that beans are deficient in methionine. Rice is also deficient in lysine but not in tryptophan. A deficiency in methionine can also be seen in corn and rice. The other amino acids deviate less from the quantities in which they appear in whole egg proteins. It must, however, be borne in mind that such a comparison can only be a rough one, because of the differences in the methods of amino acid analysis employed by Mitchell and Block for the whole egg and by the present authors for the foodstuffs we analyzed.

From the results obtained it can be inferred that beans can compensate to a certain degree for the lack of lysine in corn and rice and, on the other hand, that rice can compensate for the deficiency of tryptophan in the other two. But on a diet of beans, corn, and rice combined in the proportions in which our people consume these foods, the deficiencies of lysine and tryptophan would not be compensated for. From the information obtained from various surveys by Anderson et al. ('46) and Calvo et al. ('46) it is known that in the Mexican diet tortillas predominate in quantity over beans and rice. According to Miranda ('47), the daily intake of the average Mexican is 216.2 gm of corn, 22.5 gm of beans, and 11.6 gm of rice. This diet is not balanced with respect to lysine and tryptophan.

The results obtained with the pulque (see table 2) should be considered tentative. Since the nitrogen in this drink can vary within wide limits, determinations on a large number of samples are necessary to obtain definite figures. We can, however, say that the results show that considerable amounts of amino acids, particularly lysine, must be obtained from the large quantities of pulque that certain sectors of the population drink.

The changes produced by heat processing in different foods with respect to transformations in the composition of amino acids have been brought out as much by experiments with rats as by chemical and microbiological analysis. There is the work of Olcott and Fontaine ('41), who found that by autoclaving cottonseed meal its biological value is lessened and that by the addition of lysine the value returns to normal. Block et al. ('46) found in the same way that the decrease in biological value of a cake mix of several foods by heating is due to the transformations that occur in the lysine constituent. Greaves et al. ('38) observed that casein heated at 140°C. for 30 min. loses lysine and histidine without damage to cystine, tyrosine and tryptophan. Block et al. ('34) found by chemical analysis that the lysine content does not change materially in casein heated at 150°C. Mitchell and Block ('46) noted slight changes in the amino acid content of a corn-oats-rye mixture submitted to the procedure of "gun explosion" to obtain "rolled oats." These authors reported changes principally in the lysine (9.5%), valine (11.2%), and isoleucine (12.6%) content. However, they consider that there is not fundamentally a change in the composition of the mixture. Patton et al. ('48) analyzed casein by microbiological methods before and after heating it with glucose by reflux for 24 hours and found losses in lysine (26%), arginine (30%), and tryptophan (39%).

The present authors found (see table 1) changes resulting from the cooking of beans under the above conditions that amounted to only 9% in leucine and about 8% in lysine. Rice when cooked suffers its greatest change in its trypto-

phan content (13.3%). We consider that these changes do not essentially alter the composition of the analyzed foods from the standpoint of nutrition, notwithstanding that they are slightly beyond the range of error of the amino acid assay (±5%).

In the same manner we can see the variations that corn undergoes in passing from the raw state to that of the tortilla. These changes are principally characterized by losses in tryptophan (30%), threonine (26%), histidine (25%) and arginine (16%), and they go far beyond the range of error of the method for the amino acid assay. We did not find in the literature any reports on tortillas themselves nor on other foods that had been treated in the same way, and we believe that the observed changes, though they may seem excessive, are not produced by simple heating alone since the preparation of the tortilla involves the heating of corn with a $Ca(OH)_2$ solution for almost an hour and further cooking in the "comal" (hot plate). The changes in amino acids may be due not only to the action of the heat but in part to the action of the hot $Ca(OH)_2$ solution, possibly to partial racemization of the proteins such as Levene and Bass ('28) observed in casein heated with different NaOH solutions.

SUMMARY AND CONCLUSION

1. Nine essential amino acids were determined in corn, tortillas, beans (cooked and uncooked), rice (cooked and uncooked), and pulque, using the microbiological method.

2. Compared with whole egg, the corn, tortillas, and uncooked rice were deficient in lysine. Corn and tortillas were also deficient in tryptophan, and beans in methionine.

3. In preparing tortillas considerable changes occur in the tryptophan, histidine, threonine, and arginine content of the corn as determined by microbiological analysis, using *Streptococcus faecalis*. Beans when cooked show practically no such changes. Similarly, rice remains essentially the same on being cooked.

4. Appreciable quantities of amino acids, especially lysine, were found in pulque.

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THE VALUE OF RUTIN AND QUERCETIN IN SCURVY

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ONE FIGURE

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Rutin, the rhamno-glucoside of quercetin, became the subject of study in this laboratory following its recent large-scale isolation from buckwheat (Eskew, Phillips, Griffin, Shaines and Aceto, '48). The value of flavones and flavonols in the treatment of hemorrhagic diathesis has been summarized in Nutrition Reviews ('43, '44) and the British Medical Journal ('47). Bentsáth, Rusznyák and Szent-Györgyi ('36) reported that a beneficial effect of "citrin" on vascular permeability could be demonstrated experimentally in guinea pigs. These authors showed that guinea pigs on a scorbutogenic diet could be kept alive longer with a daily supplement of 1 mg of "citrin" than without it. From this evidence they postulated the existence of vitamin P, a substance other than ascorbic acid required by scorbutic guinea pigs.

In a later article Bruckner and Szent-Györgyi ('36) reported that "citrin" was not a pure substance but consisted of a mixture of hesperidin and eriodictyol glucoside. Bentsáth, Rusznyák and Szent-Györgyi ('37) have shown that hesperidin, or an impure sample of demethylated hesperidin, obtained from the mother-liquor of "citrin," possessed

"vitamin P" activity in scorbutic guinea pigs. They drew the general conclusion that the syndrome of experimental scurvy is due to a combined deficiency of vitamins C and P.

Zilva ('37a, '37b) repeated the work of the above authors and concluded that neither citrin, hesperidin, nor a mixture of hesperidin and eriodictyol, in doses of 1 mg, caused any delay in the onset of scurvy or the fatal termination of the disease in guinea pigs on a scorbutogenic diet. Moreover, post mortem examination of the animals confirmed this conclusion. The contradictory results obtained by Bentsáth, Rusznyák and Szent-Györgyi ('36, '37), and Zilva ('37a, '37b), have been explained by Zilva ('37b) and by Bentsáth and Szent-Györgyi ('37) as being due to the presence of traces of ascorbic acid, in amounts lower than the minimum prophylactic dose, in the scorbutogenic diet or the "citrin" used by the former workers, while the materials used by Zilva did not contain ascorbic acid. In a later article Szent-Györgyi ('38) reported that he was unable to reproduce his previous results and Moll ('38) came to the conclusion that hesperidin, among other things, did not effect the symptoms of guinea pigs on a vitamin C-free diet or on one containing a trace of the vitamin.

The present experimental work was undertaken to determine whether rutin, or its aglycone quercetin, would supplement or have a sparing action on subminimum doses of *l*-ascorbic acid in guinea pigs on a scorbutogenic diet.

GENERAL EXPERIMENTAL PROCEDURE

One hundred and seven young guinea pigs of both sexes ranging in weight from 209 to 425 gm (average, 318 gm) were divided equally, as nearly as possible, with reference to number, sex, and weight into 6 experimental groups in the following way: experiment 1, negative control group, consisted of 10 male and 9 female guinea pigs, of which 4 males and 4 females received daily 0.5 ml propylene glycol, as a control on the effect of propylene glycol; in experiment 2, 10 males and 8 females received rutin; in experiment 3, 10

males and 7 females received quercetin; in experiment 4, 9 males and 8 females received ascorbic acid; in experiment 5, 9 males and 7 females received both ascorbic acid and rutin; and in experiment 6, 8 males and 8 females received both ascorbic acid and quercetin. The animals were separated with reference to sex and kept in groups of three to 5 in screen-bottomed cages.

All guinea pigs were placed on the scorbutogenic diet described previously (Ambrose and DeEds, '47), but with the following modifications: the rolled oats were ground to about the same degree of fineness as the bran, and 3 gm of brewers' yeast and 1 gm of salt were added to each 100 gm of diet. Food and water were allowed ad libitum. One ml of U. S. P. cod liver oil was given to each animal by dropper every 5 days throughout the course of the experiment. All guinea pigs were weighed once each week. Measured volumes of all supplements were administered orally to each animal once each day. Supplements were prepared as follows: a 40% solution of rutin in propylene glycol and a 20% partial suspension of quercetin in propylene glycol were prepared fresh each week; ascorbic acid solutions were prepared fresh each day, just before administration, in glass-distilled water. Throughout the description of the experiments, "ascorbic acid" refers to *l*-ascorbic acid. Daily observations were made of all animals for symptoms of scurvy. At time of death the majority of the animals were X-rayed and post mortem examinations were made for bleeding gums or loosened teeth, hemorrhages into pleural and peritoneal cavities, hemorrhages in lungs, viscera and joints, and fragility of the long bones. Serum phosphatase determinations were made on some of the surviving guinea pigs at the close of the experiment by the method previously described (Ambrose and DeEds, '47). Of the 107 guinea pigs originally started with, only 4 animals were not considered in the evaluation of the results because they failed to withstand the rigors of the experiment for a minimum of 5 days.

RESULTS

In experiment 1, in which approximately one-half of the guinea pigs received 0.5 ml each of propylene glycol daily until death ensued, no significant difference in survival time was noted between the sexes nor between those receiving propylene glycol and those not so treated. The onset and severity of the symptoms of scurvy were essentially the same for all animals in this group. The symptoms and post mortem changes described by Cohen and Mendel ('18) for scurvy in guinea pigs were typical of our findings: tenderness, swelling, stiffening of joints, and difficulty in the use of the hind legs. Hemorrhages into gums and loosening of teeth were not observed. Some tenderness and enlargement of the costochondral region were apparent in all of these animals. On autopsy, extensive hemorrhages into the joints and fragility of the bones were consistent findings. Occasionally some petechial hemorrhages were found in the subcutaneous tissues. The viscera of practically all animals appeared normal. In the lungs of the majority of the animals there was evidence of old and recent hemorrhages which we believe were due to lowered resistance to infection. X-ray pictures of 12 of the 19 animals were taken. In all cases there was marked evidence of changes in the rib and leg bones as compared with normal animals on a non-scorbutogenic diet. In the rib bones there appeared a thin white line along the periphery of the bone with increased porosity of the rest of the bone, findings which distinctly differentiate them from the rib bones of the positive control animals. The epiphyseal line of the leg bones showed slight decrease in calcium deposition along its course, accompanied by a decrease of calcium in both the epiphysis and diaphysis. All the leg bones appeared to show evidence of fracture in the metaphyseal region. No histological studies were made of these bones.

In the animals of experiment 2, receiving 100 mg rutin daily, no significant difference in survival of the two sexes was noted. The weight loss, onset of scorbutic symptoms,

post mortem findings of hemorrhages in the viscera and joints, and fragility of the bones were essentially the same as those observed in experiment 1. The X-ray pictures taken of 16 of the 18 animals showed the same results found in the previous group.

In experiment 3, quercetin (the aglycone of rutin) was administered to guinea pigs in order to compare its effect with that of rutin alone or in combination with subminimum amounts of ascorbic acid. Accordingly, the animals were given each day 50 mg of quercetin (approximate gram-molecular equivalent of 100 mg of rutin). The difference in survival time between the sexes was not statistically significant. The weight loss, onset of symptoms of scurvy, and findings at autopsy were the same as in experiment 1 and 2. Study of the X-ray pictures obtained on 5 males and 7 females confirmed the observations on the previous groups.

It has already been pointed out that the discrepancy between the results obtained by Bentsáth, Rusznyák and Szent-Györgyi ('36, '37) and those observed by Zilva ('37b) have been explained by the latter as being due possibly to the presence of small amounts of ascorbic acid under the experimental conditions of the former workers, whereas no ascorbic acid was present in the case of Zilva's work. The experiments described thus far in the present report have confirmed this explanation, since the longevity, picture of scury, and post mortem findings for the scorbutic controls and the scorbutic animals receiving rutin or quercetin without subminimum ascorbic acid supplements were essentially identical. The succeeding experiments are, therefore, concerned with a comparison of three groups of scorbutic guinea pigs receiving supplements of subminimum amounts of ascorbic acid, and this same supplement of ascorbic acid plus either rutin or quercetin, as used in experiments 2 and 3, to determine whether the flavonoids would have a sparing or synergistic action on the ascorbic acid. Accordingly, in the three experiments that follow, ascorbic acid was administered to the guinea pigs in doses of 0.2 mg/animal/day.

In experiment 4, in which the animals were given 0.2 mg of ascorbic acid each day, 10 of the animals died before termination of the experiment on the 44th day and the remaining 7 were sacrificed. The onset of symptoms of scurvy, contrary to the observations of Zilva ('37a, '37b), appeared at about the same time as for animals in the previous experiments. The weight loss of animals in this experiment did not exceed 10% each week, whereas for the negative controls (expt. 1) the weight loss was as much as 25% and for the animals receiving rutin (expt. 2) and quercetin (expt. 3) the loss was approximately 18%. Although all the animals showed evidence of swelling and stiffening of the joints, it was our impression from gross observation that this effect was not as great as in the previous experiments. On autopsy, recent bleeding into the joints was an uncommon finding. Out of 9 animals autopsied, one showed recent hemorrhage into the joints, two showed slight or no hemorrhages, and in 6 there was evidence of absorbed hemorrhages. Two of the latter also showed evidence of recent hemorrhage. The fragility of the bones of these animals was about the same as for the negative controls. X-ray examination of the long bones of these animals also showed essentially the same results observed in the case of the negative controls. Microscopical examination of longitudinal sections of the femur or tibia, stained with silver nitrate, showed distinct changes as compared with control animals on a non-scorbutogenic diet. Calcification along the epiphyseal line seemed to have increased, as did the porosity of the epiphysis and diaphysis. In other words, there appeared to have been a redistribution of calcium in these areas rather than a loss. In harmony with this interpretation is the fact that X-ray pictures revealed fracturing in the metaphyseal region.

In experiment 5, in which each animal received daily 0.2 mg ascorbic acid and 100 mg rutin, 7 of the animals died before termination of the experiment on the 45th day. The average weight loss was slightly less than that for animals in experiment 4, but this was not considered significant. How-

ever, the general appearance of these animals was much better than that of animals in any of the previous experiments. The symptoms so easily recognized in the other experiments were much less evident in the animals of this experiment. In only a few of the animals were swelling and stiffness of joints found. On autopsy, hemorrhages, either old or new, in the joints were found even less frequently than in experiment 4. X-ray studies of the long bones and microscopic examination of longitudinal sections of the femur or tibia revealed essentially the same findings as reported in connection with experiment 4.

In experiment 6, each animal received 50 mg of quercetin per day, the same amount used in experiment 3, in addition to 0.2 mg of ascorbic acid per animal per day. The per cent weight loss of these animals, general appearance, symptoms of scurvy, and findings at autopsy were about the same as in experiment 5. The results of X-ray studies and microscopic examination of sections of femur or tibia did not differ from those of experiment 4.

Serum phosphatase studies

It has been concluded by Robison ('32) and confirmed by Gould and Shwachman ('43) that serum phosphatase activity in guinea pigs is of osteoblastic origin, and that in scurvy there is an impairment of osteoblastic activity with a concomitant rise or fall in blood serum phosphatase activity, depending on whether or not ascorbic acid supplements are administered in conjunction with the scorbutogenic diet. The present serum phosphatase studies were, therefore, made to determine whether or not such determinations might serve to demonstrate objectively that rutin or quercetin had a synergistic or "sparing action" on the small supplements of ascorbic acid used.

Serum phosphatase determinations were not made at the beginning nor during the course of the various experiments because of the large amount of blood required and the danger of loss of the animals. Therefore, determinations were

made only on animals surviving at the termination of experiments 4, 5 and 6 on the 44th, 45th and 49th days, respectively.

Serum phosphatase determinations were made on 6 of the surviving guinea pigs in experiment 4, on 8 in experiment 5, and on three of the 4 survivors in experiment 6. The values obtained, with their range, are as follows: 6.6 (5.3–8.6); 6.5 (3.4–10); and 7.0 (5.0–9.8), respectively.

As judged by our previous studies (Ambrose and DeEds, '47) and the data reported by Gould and Shwachman ('43), the values for phosphatase activity found are about one-half those for normal guinea pigs. Under the conditions of our experiments and those of Gould and Shwachman, the dose of ascorbic acid (0.2 mg per day) would tend to hold the serum phosphatase values at the level found. In other words, the administration of rutin or quercetin had no significant influence on the phosphatase activity, and the observed increase in longevity must be explained in some other way. In earlier studies (unpublished data) serum phosphatase values continued to fall in guinea pigs on a scorbutogenic diet receiving a daily supplement of 100 mg of rutin without added ascorbic acid.

Effects on longevity

The determination of serum phosphatase activity in experiments 4, 5 and 6 necessitated termination of the experiments before the survival time of all the animals had been determined. The median survival time of all the animals in experiments 1, 2 and 3 cannot be compared fairly with the median survival time of the weakest or more susceptible animals which died before termination of the last three experiments. However, it would be fair to compare the median survival time of the first 7 animals dying in each experimental group, since the groups involved approximately the same numbers of guinea pigs at the beginning of the experiments. The lowest mortality occurred in experiment 5, when the scorbutogenic diet was supplemented with a

subminimum amount of ascorbic acid plus rutin. A comparison has, therefore, been made of the median survival time of these 7 animals with that of the first 7 animals dying in each of the other experimental groups. The median survival time in the first three experiments was 12 to 15 days. In experimental groups 4 and 6, receiving ascorbic acid and ascorbic acid and quercetin, the median survival times were 20 and 22 days, respectively. In experimental group 5, receiving ascorbic acid and rutin, the median survival time was 30 days. From these data it appears that ascorbic acid and rutin, when administered simultaneously, prolonged the life of guinea pigs beyond that of animals receiving ascorbic acid, rutin, or quercetin separately, under the conditions of our experiments.

DISCUSSION

The results of the above experiments are in harmony with the conclusion of Bentsáth, Ruszynák and Szent-Györgyi ('37) that the syndrome of experimental scurvy is due to the combined deficiency of vitamins C and P. However, guinea pigs on a scorbutogenic diet without the addition of subminimum amounts of ascorbic acid (i.e., amounts less than the minimum prophylactic dose), but receiving rutin or quercetin, develop symptoms of scurvy at about the same time as guinea pigs receiving no supplements of rutin or quercetin. This is in agreement with the results of Zilva ('37a, '37b), who used citrin, hesperidin, and a mixture of hesperidin and eriodictyol, but is not entirely in accord with the claim of Bentsáth and co-workers ('36) that animals receiving citrin alone showed fewer hemorrhages than the negative controls.

When subminimum amounts of ascorbic acid were fed to guinea pigs on a scorbutogenic diet, the symptoms of scurvy were definitely less severe than the symptoms of scorbutic guinea pigs not receiving such supplements. When the scorbutogenic diet was supplemented with rutin or quercetin in addition to the subminimum dose of ascorbic acid, the symp-

toms of scurvy remained about the same as in guinea pigs receiving the ascorbic acid alone, except that bleeding into the joints was a less common finding. This observation confirms the work of Bentsáth and co-workers ('36) if we accept Zilva's explanation that traces of ascorbic acid were present under the existing experimental conditions.

Neither rutin nor quercetin fed as a supplement to guinea pigs on a scorbutogenic diet had any effect on longevity. When rutin was administered together with subminimum

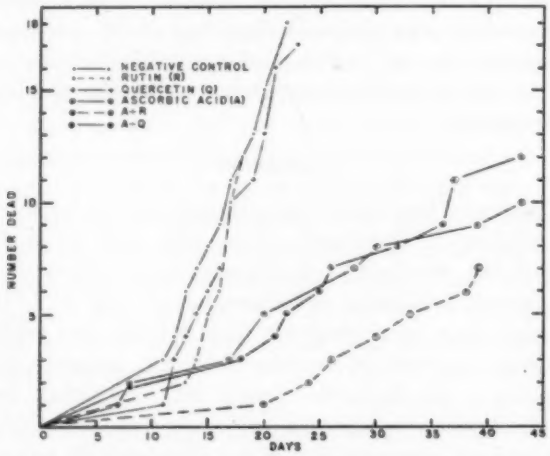

Fig. 1 The comparative effect of rutin (R), quercetin (Q), ascorbic acid (A) and mixtures of ascorbic acid and rutin (A + R), and ascorbic acid and quercetin (A + Q), on longevity in guinea pigs on a scorbutogenic diet.

doses of ascorbic acid, life was definitely prolonged. This is shown in figure 1, where it can readily be seen that rutin appears to be more effective than an equi-molecular amount of quercetin. This difference is believed to be due to the poorer solubility of pure quercetin in propylene glycol and body fluids. In preliminary studies (unpublished data) a less pure sample of quercetin extracted from goldenrod was used. This sample was more soluble in propylene glycol and the data obtained indicated that it was equally as good as

rutin, on an equi-molecular basis, in prolonging life. It is quite unlikely that the impurities of this sample contained ascorbic acid because of the extraction process used. Figure 1 shows that the first guinea pig on a scorbutogenic diet plus supplements of ascorbic acid and rutin died on the 20th day of the experiment. By this time all but three of the negative controls, 5 of the animals receiving ascorbic acid without rutin, and all but three receiving only rutin supplements and 4 receiving only quercetin supplements had died.

Since completion of this investigation a report by Cotereau, Gabe, Gero and Parrot ('48) has appeared in which the authors claim a greater storage of ascorbic acid in the liver, spleen, kidneys and adrenals of guinea pigs on a scorbutogenic diet receiving daily supplements of ascorbic acid and catechin (a mixture of epimers of d-catechin—Perrcapyl Russel) than in guinea pigs receiving ascorbic acid or catechin alone. At autopsy the scorbutic type of lesions was found in all the animals except those given ascorbic acid and catechin.

In the experiments presently reported the scorbutic lesions were less pronounced in animals receiving ascorbic acid with supplements of rutin or quercetin than in animals receiving ascorbic acid alone. However, the lesions in the animals receiving ascorbic acid alone were less severe than in the negative controls, and also less severe than in the guinea pigs receiving rutin or quercetin without ascorbic acid.

Cotereau, Gabe, Gero and Parrot ('48) concluded that storage of ascorbic acid occurs in the presence of a "sparing factor" such as catechin, and this *in vivo* protection of ascorbic acid is consistent with the observation of Parrot and Cotereau ('46) that catechin retards the rate of oxidation of ascorbic acid *in vitro*. Similarly, the present report on the value of rutin and quercetin in supplementing subminimum amounts of ascorbic acid in scorbutic guinea pigs is in agreement with unpublished data obtained in this laboratory on the antioxidant action of these flavonoids toward ascorbic acid.

SUMMARY AND CONCLUSIONS

Data are presented on the effects of rutin and quercetin in guinea pigs maintained on a scorbutogenic diet with and without the addition of subminimum amounts of ascorbic acid.

Rutin and quercetin administered orally in doses of 100 and 50 mg per animal per day, respectively, did not prolong the life of the guinea pigs beyond that of the negative control animals on the scorbutogenic diet alone. The scorbutic symptoms and the post mortem findings did not differ from those of the negative controls.

In guinea pigs receiving subminimum amounts of ascorbic acid alone, ascorbic acid and rutin, and ascorbic acid in combination with quercetin, the post mortem findings were essentially the same except for the fact that the last two groups showed fewer fresh hemorrhages than the animals receiving ascorbic acid alone. Microscopic study of stained longitudinal sections of the femurs or tibias of these three groups of animals showed essentially the same picture for all groups. This picture differed from that of positive control animals on a non-scorbutogenic diet in that the epiphyseal line showed slightly more calcification and the epiphysis and diaphysis greater porosity, which might account for the fractures in the metaphyseal region seen in X-ray pictures. The combined supplements of ascorbic acid and rutin apparently prolonged the life of scorbutic guinea pigs.

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THE EFFECT OF RETENTION OF NITROGEN IN CASEIN OR LACTALBUMIN HYDROLYSATES ON THE REGENERATION OF PLASMA PROTEINS OF PROTEIN-DEPLETED DOGS

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In previous communications (Chow, '46; Bolling, Block and Chow, '47; Chow, Seeley, Allison and Cole, '48) data were presented to demonstrate that oral administration of lactalbumin or its hydrolysate to protein-depleted dogs favors the regeneration of plasma albumin, whereas casein or its hydrolysate stimulates the production of both albumin and globulins. Since the chemical analyses of the 10 essential and a few of the non-essential amino acids showed a marked similarity in the two hydrolysates, the difference in the plasma protein regeneration properties is believed not to be due (Bolling, Block and Chow, '47) to the difference in amino acid composition alone. Hence, an explanation is yet to be found.

The superiority of lactalbumin over casein in promoting the growth of young rats (Supplee and Clark, '46) or in supporting nitrogen balance in dogs (Allison, '48) has been well demonstrated, and the difference can be compensated for by feeding the animals a higher level of casein. It is,

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therefore, of interest to ascertain whether the production of specific protein components in plasma is similarly dependent on the amount of absorbed nitrogen, which might then account for the difference in the plasma protein regeneration properties of these two milk proteins. To this end studies were undertaken which involved feeding a group of protein-depleted dogs with a casein hydrolysate at a nitrogen level which would stimulate a rapid and pronounced regeneration of plasma proteins. For comparison, three other groups of protein-depleted animals were fed three different levels of a tryptic digest of lactalbumin, so that the retained nitrogen was either greater, approximately equal to, or less than that retained by dogs receiving the casein hydrolysate. In addition, two other groups of dogs were fed the same level of casein hydrolysate as the first group but were given 1 or 2% of methionine as a supplement in order to increase the nitrogen retention (Allison, Anderson and Seeley, '47; Brush, Willman and Swanson, '47; Cox, Mueller, Elman, Albanese, Kemmerer, Barton and Holt, '47) to approximately that of the animals receiving the highest dosage of lactalbumin hydrolysate. The regeneration of various protein components in the plasma of the dogs fed these diets was measured by determining the composition of plasma electrophoretically, and the total circulating plasma proteins (the product of the plasma protein concentration and the plasma volume). The nitrogen retention was also measured for the whole period of feeding. The results are reported in the present paper.

EXPERIMENTAL

Preparation of hydrolysates

One kg of edible casein [2] was suspended in 9 l of distilled water previously warmed to 50°C. Enough 6 N NaOH (usually 85 to 90 ml) was added with continuous stirring until

[2] Borden's.

all the protein was in an even suspension and the pH remained about 7.6 for at least 30 minutes. Forty grams of a purified trypsin preparation (1:280)[3] were added and the suspension incubated at about 50°C. for 38 hours, after which time 900 gm of Celite no. 505 were added to aid filtration. The digestion was considered complete when the addition of an equal volume of a 5% trichloracetic acid solution to a small aliquot of the filtrate did not produce any opalescence. The water-clear filtrate was lyophilized. Chemical analysis showed that such hydrolysates contained about 2.0% sodium and 13.5% total nitrogen, of which 28 to 30% was liberated as amino nitrogen, as determined by the nitrous acid method.

Similarly, lactalbumin hydrolysate was prepared by suspending 1 kg of lactalbumin[4] in 9 l of water kept at 37°C. The pH was kept between 7 and 8 by continuous addition of 6 N NaOH. Forty grams of the purified pancreatic enzymes were added. During the first 6 to 8 hours the digestion took place rapidly, and the original pH was maintained by further addition of alkali. Incubation at 37°C. was allowed to proceed for 48 to 72 hours. The suspension was then adjusted to pH 5 by the addition of a 4 N HCl solution and filtered with the aid of Celite (100 gm/liter), and the filtrate was lyophylized. Chemical analysis showed that the hydrolysate contained 2.5% sodium and 12.8% nitrogen, of which 35 to 40% was liberated as amino nitrogen.

Preparation of hypoproteinemic dogs

Normal and healthy dogs were given a protein-free diet consisting of 21% sucrose, 19.4% dextrose, 32.9% dextrin, 21.8% lard, 1.8% Osborne-Mendel salt mixture, and 2.5% agar, with the following vitamins as supplements: thiamine, riboflavin, nicotinic acid, calcium pantothenate, pyridoxine, choline-2-methly-1-napthoquinone, biotin and vitamins A and D. These dogs were given daily an amount of this diet

[3] Obtained from the Wilson Laboratories.

[4] Borden 15–42.

calculated to supply 80 cal. per kilogram body weight. After a period of 6 to 8 weeks the plasma protein concentration dropped to 4.5 to 5.2 gm % and the albumin-to-globulin ratio was reduced to approximately 0.25.

Repletion with different test proteins

The protein-depleted dogs were repleted with the test diets also at 80 cal. per kilogram body weight per day at different nitrogen levels. Determinations of plasma volume (Gregersen and Stewart, '39) and the plasma protein concentrations, as well as electrophoretic analyses of plasma (Longsworth, '42) were performed before and after depletion, and also after three or 6 weeks of repletion with the test substances. It was thus possible to study quantitatively the changes of each plasma protein component during the course of protein administration. The technique of these determinations has been published elsewhere (Chow, Seeley, Allison and Cole, '48) and need not be repeated.

RESULTS

Six groups of protein-depleted dogs were fed for 6 weeks three levels of lactalbumin hydrolysate (0.35, 0.25 and 0.15 gm N/kg body weight per day) and one level of casein hydrolysate (0.35 gm N), with or without the supplementation of 1 or 2% methionine. In the 7th week the animals were given a protein-free diet. The nitrogen intake as well as excretion for the 7 weeks were determined on the basis of a weekly collection. The biological value, or the fraction of the hydrolysate nitrogen retained in the body of the test animals, was calculated according to the equation of Allison ('46): (NB)=(BV)(AN)−(EN), where (NB)= nitrogen balance, (BV)= biological value, (AN)= absorbed nitrogen and (EN)= endogenous nitrogen. It was found the digestibility of the hydrolysate diet was essentially complete. Hence, the (AN) value approached closely that for nitrogen intake (IN).

The amount of plasma protein regeneration was determined by comparing the total circulating plasma proteins before and after a given period of repletion.

TABLE 1

The levels of absorbed nitrogen and the plasma protein regeneration

PROTEIN SUPPLEMENT	NO. DOGS USED	LEVEL OF INTAKE [1]	NITROGEN BALANCE [2]	RETAINED "BIOLOGICAL VALUE" [3]	PLASMA PROTEIN NITROGEN [4]
					%
Lactalbumin hydrolysate	5	0.35	+ 0.186	0.8	4.6
Lactalbumin hydrolysate	4	0.25	+ 0.118	0.8	3.0
Lactalbumin hydrolysate	4	0.15	+ 0.072	1.0	2.7
Casein hydrolysate	5	0.35	+ 0.117	0.5	4.5
Casein hydrolysate + 1% methionine	3	0.35	+ 0.175	0.7	4.5
Casein hydrolysate + 2% methionine	3	0.35	+ 0.165	0.7	5.0

[1] Level of intake is expressed as grams nitrogen per kilogram body weight per day.

[2] Nitrogen balance is the difference between nitrogen intake and nitrogen excretion, expressed in grams nitrogen per kilogram body weight per day.

[3] "Biological value" is calculated according to the equation of Allison ('46): (NB = (BV) (AN) — (EN). (See text.)

[4] Per cent of the total absorbed nitrogen used for the synthesis of total plasma protein.

The relationship between absorbed nitrogen and plasma protein synthesis

The results given in table 1 demonstrate that all 6 diets were adequate in supporting nitrogen balance (see column 4) during the period of repletion. At the level of nitrogen intake of 0.35 gm/kg body weight per day, the lactalbumin hydrolysate was considerably more effective than the casein hydrolysate, which was improved by the addition of 1 or 2% methionine. As was to be expected, the extent of nitrogen balance was dependent on the dosages of lactalbumin hydrolysate given, being highest at 0.35 gm N intake.

The results given in column 5 of table 1 demonstrate that half of the casein hydrolysate nitrogen [5] was retained, and the retention was increased to 70% by the addition of 1 or 2% methionine. On the other hand, at least 80% of the lactalbumin hydrolysate nitrogen was retained; the retention was virtually complete when the level of nitrogen administered was reduced. The results shown in this table also demonstrate that, of the total retained nitrogen, approximately 5% was used for the synthesis of plasma protein (see column 6). This percentage was decreased to about three as the nitrogen retention was lowered.

The synthesis of plasma albumin and globulins

The difference in the plamsa protein regeneration patterns of a series of dogs fed with lactalbumin hydrolysate or with casein hydrolysate is further illustrated in table 2. The data (column 3) demonstrate that after 6 weeks of repletion dogs fed with lactalbumin hydrolysate regenerated less plasma proteins than those fed with casein hydrolysate. However, the animals in the former group showed striking increase in the albumin and essentially no increase in globulins, whereas animals fed with casein hydrolysate showed a marked increase in both the albumin and globulin fractions after 6 weeks of feeding. Supplementation with methionine for the same period resulted in a definite increase (though less marked) in the amount of globulin regenerated. The changes in A/G ratio, recorded in the last two columns of table 2, demonstrate that this ratio was the highest (0.60) for the group of dogs fed with lactalbumin hydrolysate at

[5] The biological values of the casein and lactalbumin hydrolysates obtained under our experimental conditions were considerably lower than those reported by Allison ('46). However, it must be pointed out that our data represent the average values after 6 weeks of feeding, during which time the state of protein reserves of the animals was gradually changing from that of deficiency to that of abundance. In Allison's experiments the determination was performed over a much shorter period and at such a dosage that the protein reserves of the dogs did not materially change.

TABLE 2

The effect of oral feeding of casein or lactalbumin hydrolysates at different levels of nitrogen intake on total circulating plasma proteins of dogs depleted in proteins

LEVEL OF FEEDING	NO. DOGS	TOTAL CIRCULATING PROTEINS [1]		TOTAL CIRCULATING ALBUMIN [1]		TOTAL CIRCULATING GLOBULINS [1]		A/G	
		3 weeks	6 weeks	3 weeks	6 weeks	3 weeks	6 weeks	0 weeks	6 weeks
Lactalbumin hydrolysate: 0.35 gm N/kg	4	115 ± 10.0	128 ± 10.0	185 ± 21	252 ± 23	100 ± 8.6	100 ± 9.3	0.22	0.60
Lactalbumin hydrolysate: 0.25 gm N/kg	4	121 ± 7.1	137 ± 8.7	193 ± 21	251 ± 40	106 ± 5.2	105 ± 6.5	0.23	0.46
Lactalbumin hydrolysate: 0.15 gm N/kg	4	108 ± 9.3	123 ± 7.1	176 ± 22	227 ± 16	97 ± 9.6	105 ± 7.0	0.16	0.35
Casein hydrolysate: (0.35 gm N/kg) plus 1% methionine	3	124 ± 6.1	141 ± 9.0	172 ± 23	245 ± 35	114 ± 5.7	120 ± 3.0	0.23	0.41
Casein hydrolysate: (0.35 gm N/kg) plus 2% methionine	3	133 ± 7.9	146 ± 6.1	196 ± 18	262 ± 29	116 ± 4.8	118 ± 1.0	0.26	0.44
Casein hydrolysate: (0.35 gm N/kg) alone	5	138 ± 7.1	150 ± 2.8	195 ± 7.5	210 ± 10	113 ± 10.6	136 ± 9.1	0.22	0.51

[1] Calculated as per cent of the amount determined prior to protein feeding, with standard error.

TABLE 3

The effect of oral feeding of casein or lactalbumin hydrolysates at different levels of nitrogen intake on total circulating globulins in the plasma of dogs depleted in proteins

LEVEL OF FEEDING	DOGS NO.	TOTAL CIRCULATING ALPHA (1 AND 2)[1]		TOTAL CIRCULATING GAMMA[1]		TOTAL CIRCULATING OTHER GLOBULINS[1]	
		3 weeks	6 weeks	3 weeks	6 weeks	3 weeks	6 weeks
0.35 Lactalbumin hydrolysate	4	78 ± 11.2	63 ± 5.2	73 ± 8.7	96 ± 14.3	113 ± 9.6	115 ± 16.2
0.25 Lactalbumin hydrolysate	4	64 ± 5.2	56 ± 9.6	98 ± 6.7	114 ± 10.1	135 ± 6.1	152 ± 6.3
0.15 Lactalbumin hydrolysate	4	71 ± 11.8	83 ± 15.4	81 ± 5.7	114 ± 7.2	117 ± 10.8	133 ± 4.9
Casein hydrolysate plus 1% methionine	3	90 ± 13.7	88 ± 5.4	129 ± 6.0	141 ± 13.3	135 ± 8.3	130 ± 14.7
Casein hydrolysate plus 2% methionine	3	136 ± 9.1	143 ± 15.0	93 ± 5.7	93 ± 8.3	129 ± 9.0	137 ± 9.4
Casein hydrolysate alone	5	128.7 ± 11.4	133.6 ± 12.9	106 ± 16.7	118.6 ± 20.0	130.3 ± 14.2	142.4 ± 12.2

[1] Calculated as per cent of the amount determined prior to protein feeding, with standard error.

a level of 0.35 gm N. The ratio of this group of animals approached unity when the repletion was continued for 10 weeks. On the other hand, repletion of dogs with casein hydrolysate for the same length of time did not significantly increase this ratio.

The effect of repletion with different dietary proteins on the globulin fractions was also investigated. The results (see table 3) demonstrate that the oral administration of lactalbumin hydrolysate at any one of three levels caused a marked drop in the total circulating alpha globulins. In contrast to this, the feeding of casein hydrolysate, with or without methionine supplementation, to the protein-depleted animals brought about no marked decrease and, as matter of fact, an increase of these globulins. In two cases no sharp difference in the effect of feeding these two hydrolysates on the regeneration of the gamma globulin was observed. Either hydrolysate brought about a marked increase in "other globulins," i.e., the total globulins minus the gamma and alpha globulins.

DISCUSSION

The classic studies of Rose and his associates on the physiological and nutritional properties of amino acids demonstrate the possibility of obtaining essentially normal growth in young rats by feeding the protein elements of the diet in the form of 10 pure amino acids. The removal of any one of these particular amino acids resulted in impaired growth, or in considerable losses in weight and ultimately death. More recent investigations (Wooley, '47; Womack and Rose, '46) demonstrate that in addition to the 10 essential amino acids, the presence of some unknown nutritive factors in the diet is essential for normal growth. The destruction of these factors in crude casein by acid hydrolysis or their removal by extraction with organic solvents results in suboptimum growth in rats. Relatively little attention has been directed to the possible role of these yet unidentified nu-

tritional factors which may predetermine the synthesis of a particular type of plasma protein component.

Since the difference in the plasma protein regeneration properties of casein and lactalbumin is due neither to the amino acid composition nor to the amount of the amino acid retained by the animals, it is not unlikely that nutritional factors other than the essential amino acids play a role in directing the synthesis of plasma proteins. Thus it is no longer safe to assume that the mere presence of the essential amino acids, even in great abundance, and of all the known vitamins can assure us of optimum nutrition, either for maintenance or for convalescence.

SUMMARY

Protein-depleted dogs were fed one level of casein hydrolysate. For comparison, three other groups of dogs were given different levels of lactalbumin hydrolysate so that the retained nitrogen was either greater, approximately equal to, or less than that in dogs receiving the casein hydrolysate. In addition, two other groups of dogs were administered the casein hydrolysate orally with the supplementation of 1 or 2% methionine, in order to increase the nitrogen retention to approximately that retained by the animals receiving the highest dosage of lactalbumin hydrolysate. The total nitrogen absorbed and the regeneration of various plasma protein components were determined quantitatively. The data demonstrated that the lactalbumin hydrolysate given at any one of the levels stimulated the production of albumin and led to a marked decrease in alpha globulins. On the other hand, casein hydrolysate favored the production of albumin and globulins even on supplementation with methionine, which increased the absorption of the casein hydrolysate nitrogen fed. The results, therefore, led to the conclusion that the difference in the plasma protein regeneration properties of the two protein hydrolysates is not due to the difference in the amount of absorbed nitrogen, but perhaps to the existence of an unidentified nutritional factor.

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THE ENHANCEMENT OF THE NUTRITIVE VALUE OF WHEAT GLUTEN BY SUPPLEMENTATION WITH LYSINE, AS DETERMINED FROM NITROGEN BALANCE INDICES IN HUMAN SUBJECTS [1,2]

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ONE FIGURE

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In a recent paper from this laboratory (Hoffman et al., '48) it was demonstrated that the method of Allison and Anderson ('45) for the determination of the so-called nitrogen balance index in dogs with induced hypoproteinemia could be applied to human hospital patients with protein deficiency. In that report the nitrogen balance index of a lyophilized amino acid preparation administered intravenously was found to be relatively constant in 20 subjects in spite of marked variability in their requirements of nitrogen for equilibrium. The latter values were shown to be dependent upon the nitrogen excretion on a protein free diet. This excretion varied greatly, being influenced by the degree and duration of protein deficiency, by the nature of the illness producing this deficiency, and by unknown factors. It was believed that the nitrogen balance index was a reliable gauge of the biological value of protein for human protein-deficient subjects.

[1] These studies were aided by a grant from the Interchemical Corporation, Biochemical Division, Union, New Jersey.

[2] Presented before the Federation of American Societies for Experimental Biology, March 18, 1948.

As a further extension of the Allison method, a comparison has been made of the nitrogen balance index of a specially processed gluten preparation with that of the same product reinforced with 4% lysine. The studies have been carried out on 10 hospital patients, of whom 7 had recognizable protein deficiency and three had serum protein concentrations within normal limits but might have had mild protein deficiency.

EXPERIMENTAL

As in previous nutritional investigations made in this laboratory, the nitrogen balance studies were executed in a special metabolic unit on a surgical ward of the hospital; the unit was staffed 24 hours a day by nurses specially chosen for this function. The urine specimens were collected in bottles containing toluene for each 24-hour period and were analyzed immediately. Stools were colleected in covered enamel jars, in which they were prepared for nitrogen analysis by homogenization with water and concentrated sulfuric acid, as suggested by Peters and Van Slyke ('32). Nitrogen determinations of the urine, stool and blood specimens were made by the photometric micro-Kjeldahl method described by Hoffman and Osgood ('40). These were frequently checked by the macro-Kjeldahl method utilized for the nitrogen analysis of the food samples. In the latter method, digestion was accomplished with sulfuric acid, potassium sulfate, and mercuric oxide. Sodium hydroxide with sodium sulfide were employed for the liberation of ammonia, which was distilled into a boric acid solution and titrated. As a check for completeness of the urine collection, daily creatinine determinations of the 24-hour urine specimen were made.

The basic non-protein diet consisted of a high carbohydrate drink,[3] soup made of carbohydrate, vegetable oil, and a nitrogen-poor vegetable; and a specially made protein-free candy.

[3] Made up of Cartose, provided by H. W. Kinney and Sons, Columbus, Indiana, and a beverage base.

The gluten preparations, with and without added lysine,[4] were incorporated in the diet in the form of muffins or puddings. Salt was allowed ad libitum in the soup, and was added in the preparation of the muffins and puddings. Vitamins were provded in the form of a commercial mixture,[5] 1 ml daily intramuscularly, and ascorbic acid, 150 mg daily by mouth. The total caloric intake was maintained at 35 to 40 cal. per kilogram per day. The average nitrogen content of the basic daily diet was found to be 400 mg. This quantity was not included in the calculation of nitrogen balance, since it was from poor protein sources, was constant through all the periods, and was probably of the same magnitude as the undeterminable nitrogen excretions. At worst, the error involved was small and did not affect the determination of the nitrogen balance index.

Two groups of nitrogen balance experiments were performed. In the first group of three subjects the balance studies were carried out as in the previously reported series, with progressive increases in the nitrogen intake after the nitrogen output on the basic diet alone had been determined. (In all 10 experiments an adjustment period of two days on the basic diet was allowed before specimens were collected, since it had been previously found that the nitrogen excretion in these first days was too high to be truly representative of the protein-free period.) After 5 days on gluten, the subjects were given the same quantity of gluten reinforced with 4% L-lysine for a period of 5 days. Then a large quantity of gluten was administered for another 5-day period, followed by 5 days of an isonitrogenous quantity of gluten plus lysine.

[4] These were kindly furnished by the Interchemical Corporation, Biochemical Division, Union, New Jersey. The gluten was a specially prepared powder of non-agenized wheat gluten. The *gluten-plus-lysine* was also a specially prepared powder, consisting of non-agenized wheat gluten homogeneously mixed with 4% purified L-lysine hydrochloride. This latter product is now being marketed as Elutein.

[5] Betalin Complex, kindly furnished by Eli Lilly and Company, Indianapolis, Indiana.

This alternation was continued until good positive balances were obtained with both products.

In the second group of 7 subjects the nitrogen balance experiments were carried out with only two points for the determination of the curve, on the assumption that the straight line curves demonstrated in the first three subjects would apply equally well here. In these studies, after a variable protein-free period ranging from three to 10 days, the subjects received for 8 to 10 days a quantity of gluten calculated to give a slightly negative or positive nitrogen balance. This diet then was replaced by an isonitrogenous diet of gluten plus lysine for another 10-day period. In three of the experiments it was possible to return the patients to the original gluten diet for another 10-day period.

RESULTS

The nitrogen balance findings in the first group of three subjects are shown in table 1. When the nitrogen balance is plotted against the nitrogen intake (see fig. 1), the points fall on a straight line curve both for gluten and for gluten plus lysine, as predicted by Allison. The Allison equation is $NB = K(AN) - NE_o$, where NB is the nitrogen balance, AN is the absorbed nitrogen, NE_o is the nitrogen excretion on the protein-free diet, and K is the slope of the curve or the nitrogen balance index. The nearer K approached the value of one, the higher was the biological value of the protein studied. It is possible to substitute NI, the nitrogen intake, for AN, the absorbed nitrogen, in these experiments, for the fecal nitrogen excretion was not found to be any higher during the protein feedings than during the days of the protein-free diet.

The K values for the gluten curves, which can be calculated either as $(NB + NE_o)/AN$ or as NE_o/EN where EN is the nitrogen requirement for nitrogen equilibrium, were 0.53, 0.53 and 0.59, respectively, for subjects 1, 2 and 3. The K value for gluten plus lysine was significantly higher in each of the three experiments, being 0.60, 0.67 and 0.69, respec-

TABLE 1

Nitrogen balances and nitrogen balance indices (K) in patients on diets of gradually increased quantities of gluten (G) or gluten plus lysine (GL)

SUBJECT	DIET PERIOD	NITROGEN INTAKE			NITROGEN EXCRETION		NITROGEN BALANCE		K	EN [1]
		gm/day	*mg/kg/day*	*days*	*gm/day*	*mg/kg/day*	*gm/day*	*mg/kg/day*		*mg/kg*
1	Control	0	0	3	3.49	50.2	— 3.49	— 50.2		
M.M.	G	4.52	65.1	5	5.59	80.4	— 1.07	— 15.3		
Male, 68 yrs.	GL	4.60	66.0	5	5.32	76.5	— 0.72	— 10.5		
Leg ulcer	G	6.79	97.5	5	6.34	91.0	+ 0.45	+ 6.5	0.53	94
Wt., 69.5 kg	GL	6.90	99.2	5	5.80	83.2	+ 1.10	+ 16.0	0.60	83
Tot. protein	G	9.05	130.0	5	7.30	105.0	+ 1.75	+ 25.0		
6.59 gm/100 ml	GL	9.20	132.0	5	6.95	100.0	+ 2.25	+ 32.0		
2	Control	0	0	3	1.86	42.8	— 1.86	— 42.8		
L.L.	G	2.15	49.4	5	2.88	66.2	— 0.73	— 16.8		
Female, 68 yrs.	GL	2.05	47.1	5	2.60	58.8	— 0.55	— 11.7		
Carcinoma of	G	4.30	98.8	5	3.91	89.8	+ 0.39	+ 9.0	0.53	81
uterus	GL	4.10	94.2	5	3.27	75.2	+ 0.83	+ 19.0	0.67	64
Wt., 43.5 kg	G	9.30	213.8	5	7.49	172.2	+ 1.81	+ 41.6		
Tot. protein	GL	9.02	207.3	5	6.73	154.7	+ 2.29	+ 52.6		
4.87 gm/100 ml										
3	Control	0	0	4	1.80	60.0	— 1.80	— 60.0		
N.D.	G	2.15	71.7	5	2.68	89.3	— 0.53	— 17.6		
Female, 60 yrs.	GL	2.05	68.3	5	2.44	81.3	— 0.39	— 13.0		
Amputated leg	G	4.30	143.3	5	3.57	119.0	+ 0.73	+ 24.3	0.59	103
Wt., 30.0 kg	GL	4.10	137.0	5	3.18	106.1	+ 0.92	+ 30.9	0.69	87
Tot. protein	G	9.30	310.0	5	6.58	219.5	+ 2.72	+ 90.5		
6.12 gm/100 ml	GL	9.02	301.3	5	6.28	209.4	+ 2.76	+ 81.9		

[1] Nitrogen requirement for nitrogen equilibrium.

tively. Correspondingly, the nitrogen requirement for nitrogen equilibrium (EN), which is the point where the curve crosses the zero nitrogen balance line, was 94, 81 and 102 mg per kilogram per day, respectively, for gluten. It was significantly lower in each case for gluten plus lysine, namely, 83, 64 and 87 mg per kilogram per day, respectively.

Table 2 gives the data obtained in the 7 experiments in the second group. The K values were calculated from the straight lines drawn from the point of zero nitrogen intake through the single point of gluten or gluten plus lysine feeding.

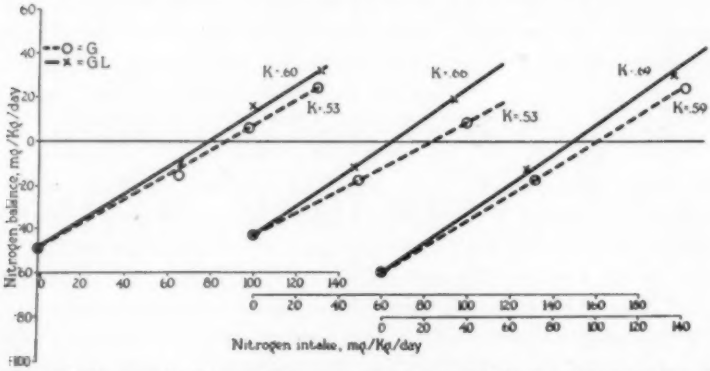

Fig. 1 Curves of nitrogen balance in relation to intake in subjects 1, 2 and 3. K in each case is the slope of the straight line curve and is the nitrogen balance index.

The nitrogen requirement for equilibrium was estimated by extension of the straight line curve through the line of zero nitrogen balance. In 6 of the 7 subjects, the K values for gluten ranged from 0.53 to 0.78, while those for gluten plus lysine ranged from 0.76 to 0.87. These values were of the same order as those in the three experiments of the first group, which indicates that no great error was introduced in drawing the rectilinear curves from only two points. In each experiment the K value for gluten plus lysine was higher than that of gluten. In the case of subject 7, the K value for gluten was 0.80 and for gluten plus lysine 0.98. Though

the values found in this case were consistent with the others in that the K for gluten plus lysine was higher than that for gluten, the absolute values were improbably high. The deviation from the mean of the K values in this case was found to be more than twice the standard deviation for both gluten and gluten plus lysine. For this reason, the values in this experiment were not utilized in the final statistical evaluation.

In subjects 4, 8 and 9, a second period of 10 days on the original quantity of gluten produced K values not appreciably different from those of the initial gluten periods. Even in the two instances in which the K value was improved in the second gluten period, this value was significantly lower than that for gluten plus lysine in the same subject. For the statistical evaluation the K and EN values for gluten in these subjects were calculated from the mean of the values from the two gluten periods.

For the total of 9 experiments in both groups the mean K value for gluten was 0.62, with a standard deviation of $\pm$ 0.09 (14.5%), and for gluten plus lysine it was 0.76, with a standard deviation of $\pm$ 0.08 (10.5%). The difference between these two means was significant, the t value [6] for statistical significance being 3.5. Similarly, for the total of 9 experiments, the mean EN for gluten was 80 mg per kilogram per day, with a standard deviation of $\pm$ 14 mg (17.5%), while that for gluten plus lysine was 65 mg per kilogram per day, with a standard deviation of $\pm$ 11 mg (17%). Again the difference between the means was significant, the t value being 2.6.

[6] The t value of the statistically significant difference between the average of two sets of experimental values was determined essentially by the method of Snedecor ('46), as follows:

$$t = \frac{a_1 - a_2}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

where a_1, σ_1, and n_1 are, respectively, the mean, the standard deviation, and the number of experiments in the first group, and a_2, σ_2, and n_2 are those of the second group. Values higher than 2.5 are regarded as indicating a significant difference in the means.

TABLE 2

Nitrogen balances and nitrogen balance indices (K) in patients on diets of gluten (G) or gluten plus lysine (GL)

SUBJECT	DIET PERIOD	NITROGEN INTAKE			NITROGEN EXCRETION		NITROGEN BALANCE		K	EN [1]
		gm/day	*mg/kg/day*	*days*	*gm/day*	*mg/kg/day*	*gm/day*	*mg/kg/day*		*mg/kg*
4										
O.N.	Control	0	0	3	2.90	46.4	— 2.90	— 46.4		
Male, 50 yrs.	G	4.80	77.0	9	4.72	75.6	+ 0.08	+ 1.4	0.61	76
Leg ulcer	GL	4.86	78.0	10	3.72	59.7	+ 1.14	+ 18.3	0.82	56
Wt., 62.3 kg	G	4.80	77.0	10	4.41	71.7	+ 0.39	+ 5.3	0.68	68
Tot. protein 7.02 gm/100 ml										
5										
G.H.										
Male, 42 yrs.	Control	0	0	4	4.30	50.0	— 4.30	— 50.0		
Leg ulcer	G	6.72	78.2	10	6.33	73.6	+ 0.39	+ 4.6	0.70	71
Wt., 86.0 kg	GL	6.72	78.2	10	5.44	63.2	+ 1.28	+ 15.0	0.83	60
Tot. protein 6.20 gm/100 ml										
6										
B.W.										
Female, 53 yrs.	Control	0	0	4	3.45	52.0	— 3.45	— 52.0		
Leg ulcer	G	4.80	72.3	9	5.73	86.3	— 0.93	— 14.0	0.53	98
Wt., 66.4 kg	GL	4.86	73.2	9	4.60	69.3	+ 0.26	+ 3.9	0.76	68
Tot. protein 7.3 gm/100 ml										

7										
E.B.										
Female, 52 yrs.	Control	0	0	10	1.86	35.0	— 1.86	— 35.0		
Morphinism	G	3.65	68.5	10	2.60	48.9	+ 1.05	+ 19.6	0.80	44
Wt., 53.2 kg	GL	3.61	67.8	10	1.92	36.1	+ 1.69	+31.7	0.98	36
Tot. protein 6.63 gm/100 ml										
8										
E.P.	Control	0	0	7	1.95	35.5	— 1.95	— 35.5		
Female, 55 yrs.	G	3.84	69.8	10	4.05	73.6	— 0.21	— 3.8	0.56	77
Parkinsonism	GL	3.78	68.8	10	3.23	58.8	+ 0.55	+ 10.0	0.76	56
Wt., 55.0 kg	G	3.84	69.8	10	3.90	70.9	+ 0.06	— 1.1	0.60	71
Tot. protein 6.02 gm/100 ml										
9										
F.F.	Control	0	0	4	2.26	51.1	— 2.26	— 51.1		
Female, 55 yrs.	G	3.07	69.3	10	3.05	68.8	+ 0.02	+ 0.5	0.74	70
Malnutrition	GL	2.99	67.5	10	2.74	61.8	+ 0.25	+ 5.7	0.87	59
Wt., 44.3 kg	G	3.07	69.3	10	3.20	72.2	— 0.13	+ 2.9	0.67	76
Tot. protein 5.86 gm/100 ml										
10										
N.L.										
Male, 38 yrs.	Control	0	0	9	2.20	42.3	— 2.20	— 4.23		
Malnutrition	G	3.65	70.1	8	3.31	63.7	+ 0.34	+ 6.4	0.78	61
Wt., 52.0 kg	GL	3.61	69.4	10	3.01	57.9	+ 0.60	+ 11.5	0.86	56
Tot. protein 4.41 gm/100 ml										

[1] Nitrogen requirement for nitrogen equilibrium.

DISCUSSION

The data from the first three balance experiments, like those from our previously reported studies, offer corroboration of the applicability of the method of Allison to human subjects with protein deficiency. The curves of nitrogen balance were rectilinear in the region of negative and low positive balance, as predicted by Allison. The K values for gluten were relatively constant, as were those for gluten plus lysine. On the other hand, the requirements of nitrogen for equilibrium were more variable, and, as in the previous studies, were dependent upon the amount of nitrogen excretion on a protein-free diet.

The findings in the remaining 7 experiments demonstrate that it is possible to obtain reasonably accurate K values for the Allison curves from only two points. The K values in 6 of these experiments were of the same order as those of the first three experiments. These results indicate that, at least for the comparison of one protein product with another in the same subject, it is unnecessary to go through the procedure of determining multiple points by a gradual increase in the nitrogen intake. Indeed, the determination of only two points may be even more reliable since, under the conditions of a relatively short experiment, the subjects can be kept on each regimen long enough for an accurate nitrogen balance assay. This scheme is essentially the same as that used by Mueller and Cox ('47), except that their experiments were carried out in normal subjects and that Allison's K values as such were not determined. Actually, one of the two points used for the curve need not be that for zero nitrogen intake; it could be that of any low nitrogen intake, provided the subjects were kept on that intake long enough for stabilization.

In each of the 10 balance experiments, the nitrogen balance index for gluten reinforced with lysine was appreciably higher than that for gluten alone. This result was of course to be expected, since numerous investigators have indicated that the limited nutritional value of wheat gluten can be enhanced by the addition of lysine. The earliest of these investigators

were Osborne and Mendel ('14). But they, as well as many more recent workers, based their conclusions on rat growth experiments, which are not necessarily applicable to man. Recently, Kuether and Myers ('48) have demonstrated the improvement in nitrogen balance in human subjects when cereal proteins were supplemented with lysine, as have Bricker, Mitchell and Kinsman ('45).

The average K value of 0.62 found in the present experiments is considerably higher than the 0.44 reported by Allison ('48) for a similar product fed to normal dogs. Allison in this communication does not report the value of the nitrogen balance index for gluten in protein-deficient dogs, but his data for other proteins indicate that the index is considerably increased when protein-depleted animals are utilized. Allison found that the K value rose from 0.44 to 0.82 in normal dogs when the gluten was reinforced with lysine; this elevation is considerably greater than that shown in the present experiments on human subjects with protein deficiency. The variance in these two sets of data may be due to species difference or possibly to an error in the K value for gluten. In both studies, at any rate, it has been demonstrated that the addition of lysine to gluten so increases the nutritive value of the product that it approaches or equals that of casein, which has been found by Allison to have a K value of around 0.80.

That the average nitrogen requirement for equilibrium using unfortified gluten was as low as 80 mg per kilogram per day was at first glance surprising. Such a value is equivalent to an intake of 35 gm of protein per day for a 70 kg person — a remarkably low requirement for what has been regarded as a relatively poor protein. However, it must be recognized that most, if not all, of our subjects were hospital patients with chronic protein deficiency. Such subjects have been shown to require a much smaller intake of nitrogen for nitrogen balance than normal individuals, unless the protein deficiency is associated with an acute "catabolic assault" such as trauma, infection, shock, or burn (Browne, Schenker and Stevenson, '44; Allison, '48; Hoffman et al., '48). The requirements for

nitrogen balance with wheat gluten feeding would undoubtedly have been much higher if the subjects had been normal and in good nutritional states. If the concentration of lysine in our wheat gluten is 1%,[7] and if the daily requirement of lysine in a 70 kg person is about 0.8 gm,[8] then the normal 70 kg subject would require about 80 gm of wheat gluten per day, or more than twice that required by our protein-deficient subjects. Such values are in keeping with the findings of Kuether and Myers ('48) and of Bricker, Mitchell and Kinsman ('45), the former utilizing whole wheat and the latter white flour.

The low requirements for nitrogen balance in our protein-deficient patients again illustrate the danger of judging the relative efficacy of any particular protein by comparing the amount required for balance in one group of subjects with that found for another protein by other investigators using a different type of subject. Nevertheless, the data imply that even a relatively poor protein is capable of producing appreciably positive nitrogen balances in severely depleted subjects, especially if properly fortified.

SUMMARY

In 10 hospital patients, most of whom had recognizable protein deficiency, a comparison was made of the nutritive value of wheat gluten alone and of the same product reinforced with 4% L-lysine.

The nitrogen balance index method, utilized by Allison in dogs, was found applicable to these human subjects. The nitrogen balance index, whether determined from the straight line curves drawn from multiple points or from only two points, tended to be constant for the particular protein preparation, whereas the nitrogen requirement for nitrogen equilibrium was more variable.

[7] Personal communication from Dr. Alfonse Walti, Interchemical Corporation, Biochemical Division, Union, New Jersey.

[8] The tentative requirement for lysine as determined by Dr. William C. Rose; personal communication.

The mean nitrogen balance index for gluten was 0.62. For gluten plus lysine, it was significantly higher, 0.76, approaching the value for casein. Thus lysine was shown to enhance the nutritive value of gluten for humans, as it had previously been shown to do for lower animals.

In patients with chronic protein deficiency even unreinforced gluten was found to be capable of producing positive nitrogen balances with moderate intakes.

ACKNOWLEDGMENTS

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INFLUENCE OF THYROID ON UTILIZATION OF VITAMIN A

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There are several reports in the literature indicating that large amounts of vitamin A have an antithyroid effect (Abelin et al., '26; Sadhu and Brody, '47; Schulze and Hundhausen, '39). The purpose of the present experiments was to test the converse hypothesis; namely, that thyroid influences the utilization of vitamin A. An experiment was set up concerned with liver storage of vitamin A in relation to thyroid activity in which it was assumed that the storage is an indirect measure of utilization; the lower the storage, the greater the depletion. If the thyroid hormone causes the destruction of vitamin A or increases the rate of its utilization, there should be decreasing vitamin A storage with increasing thyroid hormone activity.

Previously it has been indicated that adequate functioning of the thyroid gland is important for the conversion of carotene to vitamin A (Anderson and Soley, '38; Drill and Truant, '47; Escamilla, '42; Fasold and Heidemann, '33; von Euler and Klussman, '32; Johnson and Baumann, '47), although there has been some evidence to the contrary (Wiese et al., '47, '48).

Certain conclusions as to a relationship between vitamin A administration and thyroid activity seem fairly well established. These include: the delay in the metamorphosis of thyroxine-treated tadpoles to whom vitamin A is administered (Eufinger and Gottlieb, '33); the lessened elevation

of the basal metabolic rate or of oxygen consumption in animals given vitamin A simultaneous with the administration of thyroxine or dessicated thyroid (Belasco and Murlin, '40; Chevallier and Baert, '34; Sadhu and Brody, '47); and the decrease in thyroid size in animals treated with the thyrotropic hormone (Fellinger and Hochstädt, '36), thyroxine or thiouracil (Sadhu and Brody, '47) after large doses of vitamin A.

However, the literature also contains many reports concerning a vitamin A-thyroid hormone relation which are conflicting. Reports disputing any interaction were made by Baumann and Moore ('39), who found no increase in the survival rate of animals given vitamin A and thyroxine simultaneously as compared with animals given thyroxine alone. Weslaw and Wroblewski ('39) reported obtaining the same results in counteracting thyroid effects by using sesame oil as the vehicle for the vitamin. Schneider and Widmann ('34) reported that depletion in glycogen stores in the liver brought about by hyperthyroidism as a result of the administration of thyrotropic hormone was not found if carotene or vitamin A was administered simultaneously, but Fasold and Peters ('33) and Steffen and Zois ('38) reported no such effect. Finally, Johnson and Baumann ('48) were unable to find an effect of thyroid on liver storage of vitamin A.

EXPERIMENTAL

Litters of Wistar strain albino rats raised in our laboratory on the stock diet of Bills et al. ('31), varying in age from 5 weeks to two months, were used. Each litter was split into three experimental groups: (1) control, (2) thyroidectomized, and (3) thyroxine-treated[1] animals. *Thyroidectomy was chosen as the means of achieving the hypothyroid state, since no question of a side reaction from a drug*—e.g., one of the thiourea compounds—*would be involved in the interpretation of results.* Four days prior to thyroidectomy or the ad-

[1] We wish to thank Dr. S. Sidney Newcomber of E. R. Squibb and Sons for his contribution of the crystalline thyroxine used in this experiment.

ministration of thyroxine, each animal, including controls, was given 1000 U.S.P. units of vitamin A in corn oil by stomach tube (Shay and Gruenstein, '46) and placed on the standard U.S.P. vitamin A-free diet. From this time on till the conclusion of the experiment neither vitamin A nor carotene was administered, because of the concept of increased absorption from the gastrointestinal tract in hyperthyroid states (Althausen and Wener, '37) and the necessity of the thyroid hormone for conversion of carotene to vitamin A (Anderson and Soley, '38; Drill and Truant, '47; Escamilla, '42; Fasold and Heidemann, '33). The thyroidectomized animals were also given 2.5 gm calcium chloride per 100 gm of the standard diet to avoid tetany due to the inadvertent extirpation of the parathyroid glands. Crystalline thyroxine was administered subcutaneously in a dose of 0.2 mg per 100 gm of body weight per day until termination of the experiment. The litters were sacrificed at the end of a 38- to 45-day period and the livers analyzed for vitamin A and carotene. The period chosen was based on preliminary experiments. It was long enough for change in thyroid activity to have effect but shorter than the time required for complete depletion of liver storage of A in any of the groups. The pituitaries of the thyroidectomized animals were examined histologically to ascertain whether thyroidectomy had been successful (Selye, '47). In all cases where histological evidence of thyroidectomy was absent, the animals were excluded from the series. Two animals out of a total of 12 were thus excluded.

Vitamin A determinations on the livers were done by the method of Sobel et al. ('48). The statistical analysis of the results was made utilizing Fisher's ('36) test of significance for small samples in which "P" represents the probability that the difference between the two means is due to chance. When P is 0.05 or less, the difference between the means may be considered statistically significant.

DISCUSSION

From table 1 it can be seen that the mean vitamin A storage was highest in the thyroidectomized animals (43.4 ug/liver), intermediate in the thyroxine-treated animals (34.4 ug/liver) and lowest in the controls (22.8 ug/liver), while weight gain was highest in the controls (103 gm), intermediate in the thyroxine-treated animals (46 gm) and lowest in the thyroidectomized animals (36 gm); i.e. where the highest stores were obtained, the weight gain was least.

The differences in weight, comparing the control with the hyperthyroid group and the control with the hypothyroid group, were significant ($P = 0.0001$ in the former and $P = < 0.001$ in the latter), but the comparison of the weight gain of the thyroidectomized with that of the thyroxine-treated animals was not significant ($P = 0.46$). However, the differences in vitamin A storage were all significant. (P, comparing controls with thyroxine-treated animals, was 0.03, for controls compared with thyroidectomized animals was < 0.001, and for the thyroxine-treated compared with thyroidectomized animals was 0.05.)

On the basis of the results, one cannot attribute the vitamin A storage, to any great extent, to the direct action of the thyroid hormone. If this were to be the case, the thyroidectomized and the thyroxine-treated animals should show the extremes of storage and, as stated in the introduction, the hypothyroid animals should show the most and the hyperthyroid animals the least. However, a correlation can be seen in comparing storage to weight gain. The highest stores were found in the group showing the lowest weight gain, i.e. the thyroidectomized group, and the lowest stores in that group showing the greatest weight gain, i.e. the controls. It appears from these results that the presence or absence of thyroid influences storage to a large extent only to the degree that it affects growth. These results are in agreement with those of Johnson and Baumann ('48), although their experimental procedure differed in that the hypothyroid state was achieved by the administration of thiouracil and the hyper-

TABLE 1

Vitamin A content of the livers of thyroxine-treated, thyroidectomized and normal rats[1]

GROUP	LITTER[2]	SEX	WEIGHT (GM)			VITAMIN A (μG/LIVER)[3]
			Initial	Final	Change	
THYROXINE	A	F	72	138	66	43.0
		M	71	128	57	38.0
	B	M	65	121	56	28.5
		F	39	62	23	40.5
	C	F	82	138	56	30.0
		M	81	124	43	31.0
	D	F	84	119	35	36.0
		F	80	132	52	28.0
	Mean				46	34.4
THYROIDECTOMY	A	F	72	55	−17	55.0
		F	60	140	80	41.0
		M	65	144	75	45.0
	B	M	69	84	15	47.5
		F	96	134	38	37.5
		M	99	150	51	59.5
	C	F	81	127	46	33.5
		F	78	111	33	32.5
	D	F	82	62	−20	52.5
		M	81	136	55	29.5
	Mean				36	43.4
CONTROL	A	M	58	203	145	25.0
		M	33	75	42	49.0
	B	F	60	199	139	18.0
		M	47	140	93	16.0
	C	F	86	170	84	21.0
		M	91	210	119	10.0
	D	F	77	184	107	24.0
		F	92	186	94	19.0
	Mean				103	22.8
P[4]	Comparing thyroxine treated with thyroidectomized animals				0.46	0.05
P	Comparing controls with thyroidectomized animals				< 0.001	< 0.001
P	Comparing controls with thyroxine treated animals				0.0001	0.03

[1] Prior to thyroidectomy or administration of thyroxine, each animal, including controls, was given 1000 U.S.P. units of vitamin A in oil and subsequently kept on a vitamin A free diet.

[2] Each letter represents corresponding litter group.

[3] Carotene determinations were made on all livers, but the levels in all instances were negligible.

[4] P = probability that the difference between the two means is due to chance.

thyroid state by dessicated thyroid, and vitamin A was administered during the course of the experiment. On comparing weight gain and vitamin A storage in the thyroxine-treated and the thyroidectomized animals, the difference in storage is statistically significant (table 1) but the difference in weight is not. From this it appears that storage is affected in some way by the thyroid hormone; the greater the thyroid activity the lower the storage of vitamin A.

Even if one were to exclude these thyroidectomized animals which lost weight (table 1), the vitamin A stores of that group would still be greater (41.3 ug/liver) than those of the thyroxine-treated animals, although the weight gain (49 gm) would be about equal. While the difference in thyroid activity, comparing either of these two groups with the controls, might to some extent contribute to the differences in vitamin A stores, the growth effect is of so much greater influence that it completely masks any such secondary effect. Possibly, if an experiment were run using adult animals where growth effects would be minimum, this secondary effect would become more apparent. However, the secondary effect, as manifested in this experiment, is certainly not of the magnitude one would expect if the thyroid hormone influenced the destruction or utilization of vitamin A. Also, it cannot be definitely stated that this secondary effect is due to the direct action of the thyroid hormone on vitamin A, for it may be related merely to differences in metabolic activity.

SUMMARY

Vitamin A storage in the liver of rats on a vitamin A free diet was highest in the thyroidectomized animals, intermediate in the thyroxine-treated animals and lowest in the controls.

Growth of the control rats was most marked and probably accounts for the lower stores of vitamin A during a period when only endogenous vitamin A was available to the animals. Thyroid activity, in this instance, was indirect, in that growth was greatest in the euthyroid state.

Higher stores of vitamin A were found in thyroidectomized as compared with thyroxine-treated animals after a period of depletion, although there was no statistically significant difference in the weight changes. However, this cannot be necessarily ascribed to a direct thyroid-vitamin A relationship.

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VITAMIN STUDIES IN MIDDLE-AGED AND OLD INDIVIDUALS

III. THIAMINE AND PYRUVIC ACID BLOOD CONCENTRATIONS [1]

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THREE FIGURES

(Received for publication March 5, 1949)

INTRODUCTION

The data presented in this publication were collected with the purpose of establishing the fasting thiamine and pyruvic acid blood concentrations of individuals of various ages. The study comprised 220 individuals (117 men and 103 women) between the ages of 40 and 102 years. For comparison, 29 younger individuals (13 men and 16 women) were included in the investigation. The majority of the older subjects were inmates or patients in the St. Louis City Infirmary and City Infirmary Hospital, whereas the greater part of the younger subjects were private volunteers. The study was undertaken during the months from December, 1947, to May, 1948. During this period the diet offered the patients in the above-mentioned institutions was calculated to contain an average of 2,150 cal., 265 gm of carbohydrate, and 1.9 mg of thiamine daily, a vitamin content which is considered adequate with regard to the caloric intake (Cowgill, '34). The caloric value and thiamine content of the diets of the younger subjects were not evaluated. None of the individuals studied had re-

[1] Funds and materials provided by Hoffmann-La Roche, Inc.

ceived vitamin preparations prior to the blood analysis. Individuals with febrile diseases or carcinoma, and patients suffering from diabetes mellitus, diabetes insipidus, liver cirrhosis, nephritis, pernicious anemia, leukosis and organic gastric and intestinal lesions were not included in the study.

METHODS

The thiamine determinations were performed on whole blood by the method of Friedemann and Kmieciak ('43) using a sensitive fluorometer.[2] The blood was collected in the morning from the fasting and resting individuals, who had received no quinine, salicylates or other fluorescing preparations during the 24 hours preceding the thiamine determination. In the first 80 analyses duplicate blood samples were carried through the whole procedure; two aliquots of each eluate were oxidized and the thiochrome determined fluorometrically, thus giving a total of 4 readings for each blood sample. The average difference between the mean values of the two samples from the same blood was 0.25 μg%, or 7.6%, and between the two aliquots of the same eluate 0.23 μg%, or 7.0 %. After the reliability of the technique had thus been established, the subsequent analyses were performed on single blood samples with duplicate fluorometric readings on the eluate. Thiamine added to the blood was recovered quantitatively, or nearly quantitatively (92 to 103%).

Determination of blood pyruvic acid

For pyruvic acid blood determinations the procedure of Fabricius-Hansen ('48) was used, which is based on the methods of Lu ('39), Bueding and Wortis ('40), and Carlström, Myrbäck, Holmin and Larsson ('39). This procedure, which has previously been tested in the laboratory of one of the present authors, has proved its reliability and permits quantitative recovery of pyruvic acid added to the blood. The

[2] The fluorometer was constructed by the Photovolt Corporation, New York City, and was specially adapted in our laboratory for thiochrome estimations.

method is rather laborious, as usually 24 to 28 individual extraction procedures are required in each analysis for isolation of the pyruvic acid hydrazone, but several samples may conveniently be carried through at the same time.

A few modifications of the technique were found advantageous under our present working conditions. Thus, separatory funnels instead of ordinary tubes were used for separation of the layers and ether was substituted for ethyl acetate. Also a Coleman spectrophotometer was employed for the final color estimation in place of the step photometer used by Fabricius-Hansen. For a discussion of the method readers are referred to the original publication. The average difference found between duplicate analyses in the present series of determinations was 0.06 mg% or 5%.

EXPERIMENTAL

Fasting blood thiamine concentration

In figure 1 the fasting thiamine blood values are plotted graphically in relation to the age of the individuals. Table

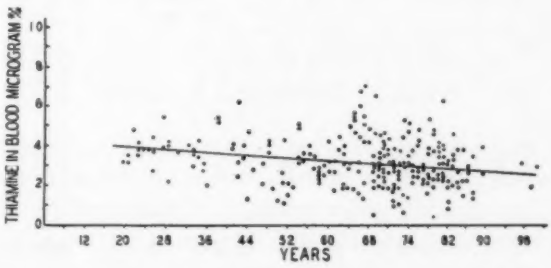

Figure 1

1 contains the summarized values for the age groups 16–39, 40–59, 60–69, 70–79, and 80–102 years, and the values for men and women separately. It will be seen from the data that the values show great variation among individuals and that there appears to be a slight tendency to a lowering of the blood values with advancing years. This is suggested by a

TABLE 1

Fasting thiamine and pyruvic acid in blood

AGE GROUP		NO.	THIAMINE IN BLOOD		S.D.[1]	OBSERVATIONS BELOW 2 μg %		NO.	PYRUVIC ACID IN BLOOD		S.D.[1]
			Mean	Range					Mean	Range	
			μg %	μg %		no.	%		mg %	mg %	
16–39	Men	13	3.8	2.2–5.3	0.7	0	0	13	1.2	0.9–1.6	0.3
	Women	16	3.8	2.0–5.5	1.0	0	0	15	1.1	0.3–1.9	0.5
	Total	29	3.8	2.0–5.5	0.9	0	0	28	1.1	0.3–1.9	0.4
40–59	Men	19	3.4	1.4–6.3	1.3	1	5	17	1.1	0.4–1.7	0.4
	Women	23	3.1	1.2–5.1	1.1	4	17	24	0.9	0.2–1.8	0.4
	Total	42	3.3	1.2–6.3	1.2	5	12	41	1.0	0.2–1.8	0.4
60–69	Men	29	3.6	0.7–7.2	1.5	3	10	18	1.2	0.5–2.8	0.3
	Women	19	4.0	1.9–7.0	1.5	1	5	17	0.9	0.5–1.5	0.3
	Total	48	3.7	0.7–7.2	1.5	4	8	35	1.0	0.5–2.8	0.3
70–79	Men	41	3.3	0.8–7.2	1.2	5	12	25	1.1	0.8–2.1	0.3
	Women	30	3.1	1.2–5.3	1.0	2	7	29	1.0	0.3–1.7	0.4
	Total	71	3.2	0.8–7.2	1.1	7	10	54	1.1	0.3–2.1	0.3
Above 80	Men	28	3.0	0.6–4.8	0.9	4	14	12	1.1	0.5–1.7	0.3
	Women	31	3.2	1.0–6.5	1.2	3	10	29	1.2	0.4–1.9	0.3
	Total	59	3.1	0.6–6.5	1.1	7	12	41	1.1	0.4–1.9	0.3

[1] Standard deviation of the mean.

moderate slope of the line representing the mean thiamine values in the different age groups in figure 1, and by the fact that 8 to 12% of the individuals above 40 years of age showed blood values below 2.0 μg%, whereas values below this level were not encountered in the younger subjects. That the tendency to a reduction of the thiamine blood value with age is only slight is exemplified by a calculation of the coefficient of correlation between age and the thiamine blood concentration, which was found to be — 0.20. The slight reduction in

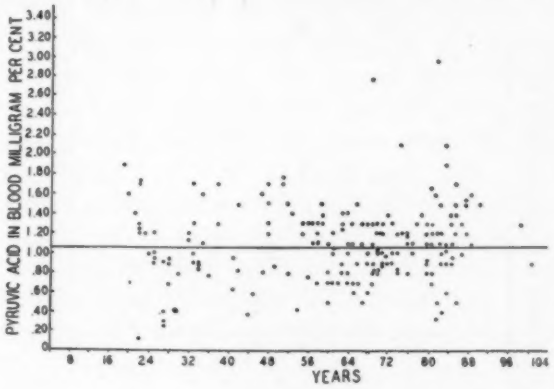

Figure 2

blood thiamine concentration may be due, in part at least, to the greater frequency of anemia with age, the red blood cells containing a higher thiamine value than the plasma. The mean thiamine blood value for the 249 subjects examined was 3.4 μg%. No difference was observed between the thiamine values in men and women.

Fasting blood pyruvic acid concentration

In figure 2 the fasting pyruvic acid blood values for 199 individuals are plotted graphically in relation to the age of the subjects. The values show a great variation among individuals but fail to reveal any effect of age or sex on the

pyruvic acid blood concentration. The mean pyruvic acid value found was 1.1 mg%.

Correlation between fasting thiamine and fasting pyruvic acid blood values

The correlation between the fasting thiamine and fasting pyruvic acid blood values was considered to be of special interest in view of observations in the literature on the occurrence of elevated blood pyruvic acid concentrations in instances of thiamine deficiency. In figure 3 the corresponding

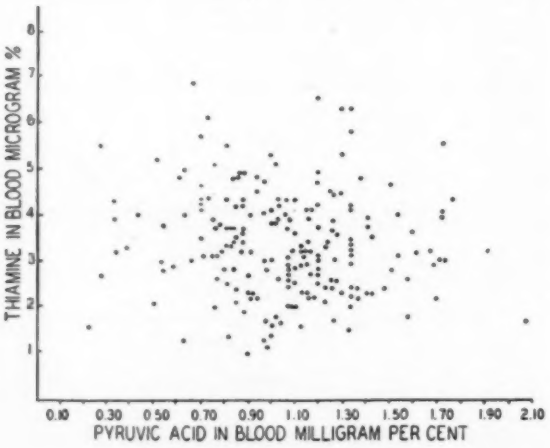

Figure 3

values have been plotted graphically. As will be seen, no definite correlation can be observed, a fact which is also revealed by a calculation of the coefficient of correlation between the thiamine and pyruvic acid values, which was found to be — 0.14.

DISCUSSION

The thiamine blood values reported in this paper are somewhat lower than the values observed by Friedemann and Kmieciak ('43) in examination of 36 adults, using the same analytical

procedure (mean value in this investigation, 3.4 μg%; in Friedemann and Kmieciak's study, 5.7 μg%), but agree quite well with the few analyses reported by Foltz, Barborka and Ivy ('44), who likewise employed the thiochrome method. As a general rule investigators using a microbiological technique have found higher values for vitamin B_1 in blood than have been observed in studies using the chemical determination, a fact which has been discussed by Hennessy ('47). Oldham, Davis and Roberts ('46), using a microfermentation procedure in an investigation of 11 young women, observed thiamine concentrations of between 4.0 and 6.7 μg%, with an average value of 5.2. The most extensive studies on this subject have been reported by Bang ('44), who examined 38 normal, non-institutionalized individuals below the age of 70, using the phycomyces growth method. The mean thiamine value observed was 7.9 μg% (5.0 to 12.4 μg%, s.d. 2.0); no certain correlation was found between the age and the blood thiamine concentration. In 13 institutionalized individuals above 70 years of age slightly lower thiamine values were encountered. These findings, apart from the higher absolute values, are in good agreement with the observations reported in the present paper.

SUMMARY

An investigation was made of the concentrations of blood thiamine and pyruvic acid in 220 middle-aged and old individuals offered a diet adequate in vitamin B_1, and in 29 younger subjects. The average concentration of thiamine was 3.4 μg% (s.d. 1.1 μg%) and of pyruvic acid 1.1 mg% (s.d. 0.3 mg%). A slight tendency was noted for the thiamine value of blood to decrease with age (coefficient of correlation age/thiamine, —0.20), whereas the pyruvic acid level showed no change with advancing years. No significant correlation was observed between the thiamine and pyruvic acid values in the same individuals (coefficient of correlation thiamine/pyruvic acid, — 0.14).

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STUDIES ON THE COMPARATIVE NUTRITIVE VALUE OF FATS

XI. ON THE POSSIBLE GROWTH-PROMOTING ACTIVITY OF $\triangle^{12}$-OCTADECENOIC ACID [1]

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A number of recent investigations have failed to confirm the reputed growth-promoting action of vaccenic acid which was postulated by Boer, Jansen and Kentie ('47). These workers presented data indicating that "summer butter" contains a substance not found in "winter butter" or vegetable oils, and which has specific growth-promoting properties when tested on rats. The compound responsible was tentatively identified as vaccenic acid ($\triangle^{11}$-octadecenoic acid), since it was believed to be identified with the $\triangle^{11}$-elaidic acid previously described by Bertram ('28). It was shown by Boer, Jansen, Kentie and Knol ('47) that a similar stimulation in growth could be produced when the diet was supplemented with vaccenic acid prepared from hydrogenated China-wood oil. The fatty acid was admittedly not a pure fatty acid, as indicated by lower than theoretical iodine number and melting point.

[1] This work was carried out under a research grant from The Best Foods, Inc. The authors wish to acknowledge the helpful advice of Professor Anton J. Carlson of the University of Chicago, Professor Arthur W. Thomas of Columbia University, and Dr. H. W. Vahlteich of The Best Foods, Inc., during the course of the experiments. Contribution number 210 from the Department of Biochemistry and Nutrition, University of Southern California.

Opposite results from those of Boer, Jansen and Kentie ('47) were obtained by Deuel et al. ('48b) when supplements of a highly purified vaccenic acid prepared by the method of Boeseken et al. ('30) were fed to rats. A specimen of hydrogenated China-wood oil also produced no stimulatory effect. It was suggested that the low growth response obtained with rapeseed oil as compared with butterfat might be partially attributable to the poor digestibility of the latter fat in rats (Deuel, Cheng and Morehouse, '48a). Euler, Euler and Linderman ('48) also reported that vaccenic acid produces no augmentation in growth of rats on a rapeseed oil or margarine fat diet. The latter workers found that growth on rapeseed oil was markedly lower than on margarine fat, just as Deuel et al. ('48b) found to be the case when the growth on a rapeseed oil diet was compared with that on cottonseed oil or butter diets. Recently, Nath et al. ('48) have further confirmed these findings by demonstrating that vaccenic acid isolated from natural sources or prepared synthetically as the *cis-* or *trans-* form causes no growth-stimulating effect on corn oil diets.

Although in this laboratory we have consistently been unable to demonstrate any superior growth-promoting activity of summer butter over that of vegetable fats (Deuel et al., '44), the possibility still exists that the results of Boer, Jansen and Kentie ('47) may have been obtained with an unsaturated fatty acid other than vaccenic acid. The latter acid might readily have been confused with $\triangle^{12}$-octadecenoic acid, which has a melting point almost identical with that of vaccenic acid (39.5°C., Richter, '42, as contrasted with 39.7–40.1°C.) and the same iodine number. Rao and Daubert ('48) have, however, obtained a value of 42.5°C. for the melting point of vaccenic acid separated from beef tallow. The importance of $\triangle^{12}$-octadecenoic acid from a nutritional standpoint is further emphasized by the fact that its presence has been reported in hydrogenated fats (Markley, '47; Ralston, '48).

The present experiments were undertaken to determine whether Δ^{12}-octadecenoic acid possesses any growth-stimulating activity. For this purpose, a sample of *trans*-Δ^{12}-octadecenoic acid was available; tests were also carried out with a *cis-trans*-mixture of Δ^{11}- and Δ^{12}-octadecenoic acids, as well as a 50–50 mixture of *trans*-Δ^{11} and Δ^{12}-octadecenoic acids. Information concerning the preparation and proof of structure of these acids is included in the appendix.

EXPERIMENTAL

The tests were carried out with weanling rats from our stock colony which were 28 days old. Littermates were evenly distributed among the 5 experimental groups. Experiments were carried out with both male and female rats. Sixteen rats (8 male, 8 female) were used in each group. The animals were kept in individual cages with raised screen bottoms. Food and water were given ad libitum but the amount of food consumed was measured. Diets were prepared weekly and were fed on alternate days. The reserve food was kept in the refrigerator between feedings.

The diets were similar to those used earlier in the vaccenic acid tests (Deuel et al., '48b). The basal diet consisted of ground whole wheat, 72 parts; commercial casein, 5 parts; yeast,[2] 10 parts; salt mixture (Osborne-Mendel, '17), three parts; and fat,[3] 10 parts. The fats used, and the supplements for the various experimental groups, were as follows: group I, butterfat [4] and no supplement; groups II to V, cottonseed oil [5] with no supplement (II), with 20 mg of Δ^{12}-*trans*-octadecenoic acid (III), with 40 mg of a mixture of Δ^{11}- and Δ^{12}-*trans*-octadecenoic acids (IV), and with 40 mg of a mixture of *cis*- and *trans*-Δ^{11} and Δ^{12}-octadecenoic acids (V). The

[2] Anheuser-Busch, strain G.

[3] The following supplements were added to all oils per 100 gm oil: carotene (General Biochemicals, Inc.) 2.4 mg; fish liver oil containing 350,000 I.U. of vitamin A per gram, 19.2 mg; crystalline vitamin D_3, 0.05 mg; α-tocopherol, 36.0 mg; commercial butter flavor, 1.2 mg.

[4] Knudsen brand, sweet cream butter, obtained locally.

[5] Wesson oil.

TABLE 1

Summary table of mean starting weights, weight gains, and food consumption of rats receiving a diet containing butterfat (I) cottonseed oil (II), cottonseed oil plus trans-Δ^{12}-octadecenoic acid (III), cottonseed oil plus Δ^{11}- and Δ^{12}-trans-octadecenoic acids (IV), or cottonseed oil plus trans- and cis-mixture Δ^{11}- and Δ^{12}-octadecenoic acids (V). Each group contained 16 rats (8 males, 8 females).

DIET NUMBER	STARTING WEIGHT AT 28 DAYS OF AGE			GAIN DURING 7 WEEKS			FOOD INTAKE IN 6 WEEKS		
	Male [1]	Female [1]	Average	Male [1]	Female [1]	Average	Male	Female	Average
	gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
I	47.9 ± 2.1	50.3 ± 2.3	49.1	175.3 ± 8.3	124.3 ± 5.5	149.8	581.9	553.3	567.6
II	49.0 ± 2.6	49.9 ± 2.6	49.5	181.9 ± 9.6	119.1 ± 3.3	150.5	569.2	515.1	542.2
III	50.9 ± 2.6	48.3 ± 2.4	49.6	169.5 ± 12.0	119.8 ± 4.1	145.7	565.9	525.3	546.6
IV	49.6 ± 2.0	47.3 ± 2.2	48.5	182.5 ± 16.5	123.0 ± 4.6	152.8	586.7	499.9	543.8
V	49.1 ± 2.5	51.8 ± 2.9	50.5	181.6 ± 8.7	110.1 ± 3.9	145.9	611.1	527.6	569.4

[1] Including the standard error of the mean calculated by the formula $\sqrt{\Sigma d^2/n-1}/\sqrt{n}$, where "d" is the deviation from the mean and "n" is the number of observations.

supplements were dissolved in cottonseed oil so that the desired amount was present in 0.1 ml, and were administered to the rats daily.

The results for the 5 groups of rats are summarized in table 1.

DISCUSSION

No stimulating effect was noted on the growth of rats when Δ^{12}-octadecenoic acid or a mixture of Δ^{11}- and Δ^{12}-octadecenoic acids were administered to animals receiving a cottonseed oil diet. This would indicate that no special growth-promoting action can be ascribed to the unsaturated Δ^{12}-octadecenoic acid. The result of Boer, Jansen and Kentie ('47) apparently cannot be due to the presence of the Δ^{12}-octadecenoic acid in butterfat, which might readily have been an unsaturated acid mistaken for vaccenic acid.

Turpeinen ('38) also carried out tests with the ethyl ester of Δ^{12}-octadecenoic acid prepared from 12-hydroxystearic acid. When 100 mg of a 1:2 mixture of the *cis*- and *trans*-isomers were fed daily to rats on a fat-free diet, no cure for the "fat deficiency" syndrome could be noted; Δ^{12}-*cis*-octadecenoic acid was also found to be ineffective in curing this deficiency when fed in 100 mg doses.

The present experiments also confirm the earlier ones of Deuel et al. ('44, '48a, '48b) in showing that an identical growth obtains when the dietary fat is butterfat or cottonseed oil. Thus the average gain in weight over the 7-week period was 149.8 gm with the butterfat group and 150.5 gm with the cottonseed oil group. These experiments would seem to answer in the negative the question as to whether Δ^{12}-octadecenoic acid possesses any specific nutritive properties. They further confirm this negative answer by demonstrating identical growth on butterfat and cottonseed oil diets.

SUMMARY

1. No increase in growth over that obtained with the unsupplemented diets could be noted in rats fed diets containing cottonseed oil and supplemented with Δ^{12}-*trans*-octa-

decenoic acid, a 1:1 mixture of $\triangle^{11}$- and $\triangle^{12}$-*trans*-octadecenoic acids, or a mixture of *cis*- and *trans*-$\triangle^{11}$- and $\triangle^{12}$-octadecenoic acids.

2. Identical growth was obtained whether the diets contained summer butterfat or cottonseed oil.

3. The tests further confirm the inactivity of vaccenic acid ($\triangle^{11}$-octadecenoic acid) as a dietary supplement.

APPENDIX

Preparation of acids (Gooding and Brown)

The acids to be described were prepared with the intention of comparing them with the solid unsaturated acids of butterfat. It was also our intention to identify more positively the acid of butter, since it appeared that vaccenic acid might have been confused with the closely similar *trans*-12-octadecenoic acid in view of the poor degree of purity reported for the naturally-occurring acid. This latter objective has not been achieved, since we have been unable to resolve contradictory results of oxidation and ultimate analyses. However, we may note that the acids from butter are of the *trans* configuration, since their infrared absorption spectra are very simliar to, if not identical with, that of vaccenic acid isolated from beef tallow and reported by Rao and Daubert ('48), and also that of synthetic vaccenic acid reported by Ahmad, Bumpus and Strong ('48). The absorption curve of the butter acid is also closely similar to that of our $\triangle^{12}$-octadecenoic acid described below.[6]

From Texas farm butter there was obtained by means of lead salt and mercury salt methods, combined with distillation and crystallization from MeOH, a material of m.p. 35–35.5°C., I.V. 85.0 and eq. wt. 287.5, and from New York dairy butter a material of m.p. 34.7–36.3°C. and I.V. 84.0. Repeated crystallization of the latter product (10 gm) from acetone gave 1.7 gm of acid of m.p. 41–41.5°C. and I.V. 86.2. Ahmad et al. ('48) have reported m.p. 43–44°C. and I.V. 87.3 for synthetic vaccenic acid.

One thousand grams of hydrogenated castor oil (I.V. 1.7) was dehydrated (Fokin, '12) under reduced pressure by heating for three hours at 260°C. with 1% naphthalene sulfonic acid. The resulting product was converted to methyl esters by methanolysis, water-washed, dried and distilled. The ester mixture of I.V. 69.0 was

[6] We are indebted to Dr. H. T. Beans, Professor Emeritus of Columbia University, for the determination of the infrared absorption spectra on these compounds.

twice partially solidified by chilling to decrease the stearate and OH-stearate content, by which means the I.V. of the final filtrate was increased to 75.1.

The liquid esters were saponified with KOH, neutralized with glacial acetic acid, and the lead salts insoluble in SD 30 alcohol were filtered by suction using a rubber dam. The lead soaps were washed with cold water before being converted by boiling with 1:1 HCl to acids of 70.4 I.V. The Bertram mercury salt method was used to separate the solid unsaturated acids from the saturated acids. The acids recovered from the filtrate, designated as *trans*-12-octadecenoic (I.V. 85.0, m.p. 33.5–37.5°C., S.V. 199.0, A.V. 172), were oxidized by $KMnO_4$ in acetone. The dicarboxylic acid fraction had an eq. wt. of 116 [theoretical for $(CH_2)_{10}(COOH)_2$, 115] and a m.p. of 94–101°C. A later purification by crystallization from diluted acetone increased the I.V. to 87.3°C. and the m.p. to 40–41°C.

A mixture of *trans*-11 and *trans*-12 C_{18} acids was prepared by isomerization of the liquid unsaturated acids recovered from the filtrate from the first lead soap separation following the dehydration described above, by heating with 0.5% Se powder for 5 hours at 210°C. Solid unsaturated acids were separated from liquid unsaturated acids by the lead soap method. The distilled, bleached, and steam-deodorized solid unsaturated acids had the following values: I.V. 86.7, m.p. 34.5–36.0°C., A.V. 198.7, and S.V. 198.8. A portion was oxidized to yield a dicarboxy acid of m.p. 96–103°C. and eq. wt. 110.5, corresponding to that which result from the oxidation of a mixture of approximately 65% of Δ^{11} and 35% of Δ^{12} acids.

The dehydration of hydrogenated castor oil was repeated and the methyl esters distilled and saponified to obtain a mixture of acids of I.V. 75.0, A.V. 189, S.V. 198.7. This product was assumed to be a mixture of *cis*- and *trans*- Δ^{11} and Δ^{12} acids, with a small proportion of stearic acid and corresponding methyl esters.

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STUDIES ON THE COMPARATIVE NUTRITIVE VALUE OF FATS

XII. THE DIGESTIBILITY OF RAPESEED AND COTTONSEED OILS IN HUMAN SUBJECTS [1]

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Variations in the digestibility of several fats in rats as compared with man have recently been reported. Thus, the coefficient of digestibility of hydrogenated peanut or corn oils melting at 50°C. was found to be 92.0 and 88.5, respectively, in man, although a higher melting hydrogenated peanut oil (m.p., 52.4°C.) had a digestibility coefficient of 79 (Holmes and Deuel, '21; Deuel and Holmes, '22). Blended hydrogenated cottonseed oil melting at 50°C. was digested to the extent of 87%, while blended hydrogenated peanut and corn

[1] This work was done under a contract between the University of Southern California and the Research and Development Branch, Military Planning Division of the Office of the Quartermaster General, U. S. Army.

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Contribution no. 209 from the Department of Biochemistry and Nutrition, University of Southern California.

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[3] We wish to thank Mr. W. R. Harriman, Director, and Dr. Douglas W. Ritchie, Medical Director, Rancho Los Amigos, for making arrangements for this study.

oils melting at 51.1 and 54°C. were found to be digested to the extent of 92.8 and 91.5%, respectively, in tests on human subjects (Deuel and Holmes, '22). In distinction to these results on man, hydrogenated cottonseed oil melting at 46°, 54°, and 65°C. was found to be digested in the rat to the extent of only 83.8, 68.7, and 24.0%, respectively (Augur et al., '47). Hydrogenated lards melting at 55° and 61°C. had digestibility coefficients of 63.2 and 21.0, respectively (Crockett and Deuel, '47).

Another example of divergent results between man and rats has been noted with rapeseed oil. Although Holmes ('18) reported a digestibility coefficient of 98.8 for 4 male subjects who consumed an average of 82 gm of rapeseed oil daily, Deuel et al. ('48) found that crude rapeseed oil was digested only to the extent of 77% and that the refined oil was 82% utilized by rats.

There are several possible explanations for these apparent discrepancies between fat digestibility in man and rats. The first suggestion is that a species difference actually exists. An example of species variability which is well known is in the response to castor oil. In distinction to the cathartic action produced by castor oil in man, the rabbit, the guinea pig, and sheep are able to utilize this fat to a high degree and no intestinal upset results (McCay and Paul, '38; Paul and McCay, '42).

A second possibility in explaining the variable results is that different procedures were employed in the tests. In the experiments on man, account was taken only of the ether-soluble fraction of the feces, which would include the neutral fatty, fatty acid, and nonsaponifiable components. In the tests on rats, the fat excreted as soaps was also considered. The inclusion of the soap fraction does not change the calculated digestibility of such low-melting fats as margarine fat, commercial hydrogenated cottonseed oil,[4] prime steam lard, and bland lard since the soap content is low and the correction for metabolic soap compensates for this factor.

[4] Crisco.

However, this is not the case with the higher melting fats. Without considering the soap fraction, a hydrogenated lard sample melting at 55°C. was calculated to be digested to the extent of 97.6%; actually, when the soap figure was included, the fat was found to be utilized only 63.2% (Crockett and Deuel, '47). In another hydrogenated lard sample melting at 61°C., these same authors report a coefficient of digestibility of only 21; had the soap fraction not been considered, the calculated value would have been 88.7.

The present experiments were undertaken to determine whether rapeseed oil actually is as completely digested in man as the earlier tests indicate or whether the inclusion of the soap fraction of the feces in the computation would alter the conclusions. Cottonseed oil was included for comparison as it is an oil generally considered to be highly digestible in man and one which has been reported to be highly utilized by rats.

EXPERIMENTAL

The experiments were carried out on 8 volunteer subjects who were patients at the Rancho Los Amigos, which is one of the units for the chronically ill of the Los Angeles County Hospital System. Some of the subjects were the same individuals who had served in earlier studies where a comparison of the nutritive value of fresh and dehydrated foods was made (Deuel et al., '47). They were not suffering from any ailments which would be expected to alter their digestive processes or their nitrogen metabolism. The subjects were confined to a single ward which was used exclusively for the test. The other conditions of the test are similar to those reported earlier (Deuel et al., '47).

Three different menus were employed, which were repeated in rotation over the experimental periods. They were identical in the rapeseed and cottonseed oil tests except that the oil used in the food preparation varied with the oil under consideration. The composition of the menus and the amount of each food served are included in table 1.

About 88% of the fat consisted of the test fat, and the balance was made up of the lipid components of the low-fat diet. The average daily protein intake was calculated at 66.0 gm and of fat at 58.4 gm, of which 51.6 gm was the dietary fat under investigation. The average caloric value of the diets

TABLE 1

The composition of the different menus employed in the fat digestibility tests

BREAKFAST		LUNCH		DINNER	
Food	Amt.	Food	Amt.	Food	Amt.
	gm		gm		gm
		Menu I			
Strained orange juice	183	Mashed potatoes	166	Vegetable soup	157
Cornflakes	16	Gravy	65	Cottage cheese	100
Graham date gems	89	String beans	150	Peach (half)	100
Sugar	22	Hot rolls	58	Lettuce	20
		Lettuce with French dressing	50 10	Mayonnaise Bread	15 30
		Apricot tapioca	161	Prune whip	112
		Menu II			
Strained orange juice	183	Spanish rice	176	Cottage cheese	100
Rice Krispies	30	Glazed carrots	113	Apricots	40
Cinnamon rolls	65	Cornbread	65	Orange	60
Sugar	22	Lemon sponge pudding	116	Pear in syrup	60
				Lettuce	20
				Mayonnaise	10
				Muffins	53
				Brown sugar tapioca	112
		Menu III			
Prunes	100	Chili and beans	207	Creamed peas on	164
Cornflakes	16	Raw chopped onions	20	Steamed potatoes	150
Bread	48	Bread	48	Sliced tomato	100
Sugar	18	Lettuce	50	Cornbread	90
		Beets	30	Pear in Jello	152
		Celery	25		
		Green pepper	8		
		French dressing	10		
		Shortcake	70		
		Cherries	50		

Additional food served: all meals — skimmed milk, 200 gm; breakfast — black coffee, 180 gm.

was 2,028 cal. and the daily food average 2,141 gm. One subject (Brown) received one-fourth additional portions in both tests, while the remaining 7 subjects received the quantities of foods listed in table 1.

The diets employed were extremely appetizing and were eaten with considerable relish. It was believed that such a dietary regime is more natural and avoids the considerable monotony that was the case with the blanc mange diets employed in the extensive series of tests at the U. S. Department of Agriculture (Langworthy, '23). Excellent subject cooperation can be obtained over long periods of time by the use of such a varied diet schedule. It does have the objection that small amounts of other fats are included in the basic diet. However, since these are apparently almost completely digested, they are not believed to influence the behavior of the predominant fat. The rolls, muffins, cornbread, shortcake, and gems had a considerable amount of the experimental fat incorporated in them, as did the French and mayonnaise dressings. These foods were as acceptable irrespective of whether rapeseed or cottonseed oil was used in their preparation.

The rapeseed oil was a commercial sample [5] which was refined by bleaching and deodorizing.[6] The refined oil had a light yellow color, a satisfactory odor, and a free fatty acid content of only 0.01%. The following constants were also found: saponification number, 167.7; iodine number, 96.3. It was the same product employed in the earlier tests on rats (Deuel et al., '48). The cottonseed oil used was a commercial oil.[7]

The methods of urine analysis were similar to those employed earlier (Deuel et al., '47), while the estimation of the fecal fat (neutral fat, fatty acid and soap fractions) was similar to that employed by Augur et al. ('47). No markers were used in the separation of feces. Since the subjects

[5] Obtained from the Pacific Vegetable Oil Company through the courtesy of Mr. M. G. Barradas of The Best Foods, Inc., San Francisco, California.

[6] We wish to thank Mr. R. H. Neal of the Bayonne Laboratories of The Best Foods, Inc., for refining the rapeseed oil.

[7] Wesson.

were accustomed to having a bowel movment each morning, the stool sample on the day of the test was rejected but the remaining stool samples during the experiments, including that excreted on the morning following the termination of the diet, were used for analysis. Because of the relatively long period of the test on each fat (9 days), the inaccuracy due to separation is considered to be minimum. The amount of fat consumed was estimated on the basis of the amount used in the preparation of the food.

The calculation of the digestibility coefficient was made by the usual procedure, making allowance for metabolic fat. Instead of using the correction factor of 9.89% of the weight of the dried stool (Langworthy and Holmes, '15), which is based only on the metabolic neutral fat, fatty acid, and non-saponifiable components excreted, a new figure of 19.8% was employed for total metabolic lipids. This was calculated on the assumption that the neutral fat, fatty acid:soap ratio was the same as the ratio between these substances in the current tests. The soap makes up about 50% of the total fecal fat in the 16 tests reported here.

RESULTS

The results for the rapeseed oil tests are included in table 2, while those for the cottonseed oil experiments are given in table 3.

The average digestibility of rapeseed oil was 99.0%, while the figure for the cottonseed oil was 96.5%. Seven of the 8 subjects consumed 525.6 gm of fat over the 9-day period, of which 464.8 gm consisted of the fat under investigation. The 8th subject, who consumed 25% more of the diet, ate 657 gm of fat, of which 580 gm consisted of the fat under investigation. No nausea or other unpleasant symptoms were noted during the course of the investigation.

The data on nitrogen excretion indicate that both fats are without deleterious effect on the nitrogen metabolism. The urinary nitrogen is identical on the rapeseed and cottonseed oil diets (8.62 gm) while the fecal nitrogen values are also

TABLE 2

Summary of the digestibility experiments on human subjects on a natural diet containing rapeseed oil as the principal fat component

SUBJECT	CALORIC INTAKE	NITROGEN/GM/DAY				CREATININE	URINE VOL.	FECES GM/DAY		FAT IN GM/DAY				COEFFICIENT OF DIGESTIBILITY
		Food	Urine	Fecal	Balance			Moist	Dry	Food [1]	EXCRETED			
											Neutral fat, fatty acids	Soaps	Total corrected	
						mg	*ml*							
Brown	2,500	12.94	8.53	0.77	3.64	1255	2295	72.0	13.3	73.0	1.28	0.72	0.00	100.0
Belloni	2,000	10.36	7.84	0.74	1.78	1659	1936	70.0	16.0	58.4	1.87	1.07	0.00	100.0
Keeper	2,000	10.36	8.54	0.31	1.27	1261	2047	29.4	7.8	58.4	1.10	0.84	0.40	99.4
Kingston	2,000	10.36	7.98	0.73	1.65	1666	1466	144.2	15.9	58.4	1.39	1.17	0.00	100.0
Burns	2,000	10.36	8.66	0.76	0.94	1551	1761	65.3	16.6	58.4	1.98	1.09	0.00	100.0
Oliver	2,000	10.36	7.42	0.90	2.02	1184	1583	59.2	17.3	58.4	2.26	1.12	0.00	100.0
Karrer	2,000	10.36	9.26	1.09	0.01	1546	1769	96.7	21.4	58.4	2.96	2.78	1.50	97.4
Butler	2,000	10.36	10.77	0.54	0.05	1865	1922	75.6	20.8	58.4	3.92	3.73	3.53	94.0
Average		10.68	8.62	0.73	1.33	1498	1847	72.8	16.1	60.2	2.10	1.56	0.68	99.0

[1] Estimated intake from quantity added to the food.

TABLE 3

Summary of the digestibility experiments on human subjects on a natural diet containing cottonseed (Wesson) oil as the principal fat component

SUBJECT	CALORIC INTAKE	NITROGEN/GM/DAY				CREATININE	URINE VOL.	FECES GM/DAY		FAT IN GM/DAY				COEFFICIENT OF DIGESTIBILITY
		Food	Urine	Fecal	Balance			Moist	Dry	Food [1]	EXCRETED			
											Neutral fat, fatty acids	Soaps	Total corrected	
						mg	*ml*							
Brown	2,500	12.68	8.48	0.70	3.50	1422	2441	63.9	12.9	73.0	1.00	2.79	1.23	98.4
Belloni	2,000	10.12	7.93	0.93	1.26	1686	1828	81.0	21.7	58.4	3.00	3.06	1.76	97.2
Keeper	2,000	10.12	9.05	0.61	0.46	1517	1954	27.5	13.3	58.4	5.32	2.10	4.78	91.8
Kingston	2,000	10.12	7.83	0.74	1.55	1833	1546	96.9	15.7	58.4	1.47	2.68	1.04	98.2
Burns	2,000	10.12	9.16	0.42	0.54	1737	1869	70.0	19.9	58.4	2.07	3.35	1.48	97.5
Oliver	2,000	10.12	7.79	0.81	2.52	1242	1609	55.7	15.9	58.4	1.41	2.72	0.98	98.3
Karrer	2,000	10.12	9.15	0.91	0.06	1647	1824	82.1	19.6	58.4	1.77	3.90	1.79	96.9
Butler	2,000	10.12	9.55	0.56	0.01	1777	1604	56.4	17.5	58.4	3.82	3.22	3.57	94.0
Average		10.44	8.62	0.71	1.11	1608	1834	66.7	17.1	60.2	2.48	2.98	2.08	96.5

[1] Estimated intake from quantity added to the food.

practically the same (0.73 and 0.71 gm). An equally favorable positive nitrogen balance obtains in tests with both oils (1.33 and 1.11).

DISCUSSION

The present tests indicate that rapeseed and cottonseed oil are almost completely digested in man when one includes in the calculation that portion excreted as soap. The figure for rapeseed oil (99.0) is practically identical with the original value of Holmes ('18) of 98.8, where the soap fraction was not considered. It indicates that a species difference does exist between man and the rat with respect to utilization of rapeseed oil.

Although it is recognized that a comparison of digestibility in rat and man is complicated by the different diets employed in the two cases, such a variation does not bring about a difference in the digestibility of cottonseed oil, but only of rapeseed oil. This would seem to preclude the difference in dietary regime as being responsible for the variation in the digestibility of rapeseed oil in rats and man.

The coefficient of digestibility reported here for cottonseed oil (96.5) also agrees well with earlier reports where the soaps were not taken into consideration. Thus, Langworthy and Holmes ('17) give a value of 97.6 for normal man, while Holt et al. ('35) have reported a figure of 96.9 for normal infants.

The differences in utilization between rapeseed and cottonseed oils, while quite small, would seem to be real from a statistical viewpoint. Thus, in no single instance is there any overlapping of the results of individual tests with cottonseed oil and the average value noted in the rapeseed oil tests. This seems to be largely the result of a higher soap excretion in the cottonseed oil tests as compared with the rapeseed oil experiments (2.98 vs. 1.56 gm). Inasmuch as the diet used is relatively high in calcium, it is also possible that the bivalent ion may have a greater depressing effect on the fatty acids of cottonseed oil than it does on the fatty acids of rapeseed oil (Cheng, Morehouse and Deuel, '49).

The present tests have shown that a low fat diet can be used as a basis for such fat studies, and can be made an extremely appetizing one by incorporation of considerable amounts of the fat under study. Such a procedure probably has advantages over the largely artificial diets formerly employed in such studies in that the ration is tolerated over long periods of time and allows studies to be made of the availability of fats when they are incorporated into a normal diet.

SUMMARY

1. The digestibility of rapeseed oil has been found to be 99% in normal men, while that of cottonseed oil was found to be 96.7% when the excretion of soap is also considered.

2. While the differences in values between these fats are only slight, they are apparently significant. They result from the somewhat greater excretion of soap in the cottonseed oil tests.

3. The results using rapeseed oil prove that a species difference obtains in respect to digestibility between man and the rat.

4. A basic diet containing a minimum quantity of fat was employed which avoids monotony and provides an appetizing and varied menu.

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NUTRITIVE PROPERTIES OF PROTEIN IN DIFFERENT CUTS OF BEEF

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In view of the high price of beef, there is need for information concerning the nutritive properties of the meat of one cut as compared with those of another. Since protein is the most important nutritive constituent of lean beef, the question arises as to whether the protein in the less expensive cuts, such as the neck, shank, or chuck, is of as high nutritive value as that in the higher-priced cuts, such as the round, loin or rib. Published results of research on meats do not supply an adequate answer.

The results of earlier investigations suggest that the biological value of the protein in different cuts of beef is materially influenced by the proportion of connective tissue present (Mitchell, '27). Much work has been done by Mitchell and associates ('27, '28) and by others to develop an accurate method for the estimation of collagen in meats, but the results have not been entirely satisfactory. Mitchell et al. ('28) reported that the eye muscle of the rib contained the lowest percentage of collagen, whereas the round, sirloin and porterhouse cuts showed slightly higher proportions. The fore shank contained much more collagen than the rib eye muscle.

The quantitative determination of each of the amino acids essential for the rat, and of cystine, is of fundamental importance in a comparison of the nutritive values of the pro-

tein in different cuts of beef, but the final proof must depend upon the results of animal experimentation. Kraybill ('48) measured the amounts of 10 essential amino acids and cystine in the rib, chuck, neck, plate, rump and flank cuts of both choice and utility grades of beef and reported that significant differences were not found. He suggests that "The methods for estimation of the amino acids may not be sufficiently accurate to determine the small differences existing in these cuts." Kuiken et al. ('48) found that all 10 of the essential amino acids in roast prime ribs of beef are completely available to the rat.

The purpose of the experiments reported in this paper was to determine, by means of feeding tests with young albino rats, the relative growth-promoting values of the protein in the round, loin, rib, chuck, neck and shank cuts from each of two carcasses of beef of different ages. Dried skim milk and mixtures of beef and gelatin were tested for comparative purposes.

EXPERIMENTAL

Products tested[1]

Cuts from the carcasses of two beef cattle, a young steer and an old cow, were used in the experiments. A brief history of each follows:

(1) A young beef-type Shorthorn steer was weaned at approximately 8 months of age, when it weighed 508 lb. and was graded "low choice" as a feeder. The steer was fed individually for 220 days, the grain mixture consisting of corn 5 parts, oats 3, bran 1, and linseed oil meal 1 part, by weight. Number 2 leafy alfalfa hay was fed as roughage. At the end of the fattening period the steer weighed 905 lb., having made an average daily gain of 1.8 lb. The steer was graded "low choice." The chilled carcass weighed 534

[1] The authors acknowledge, with thanks, the assistance of R. L. Hiner in the slaughter of the animals and the preparation of the cuts for experimental purposes.

lb. and was graded "high good." The fat content and distribution were normal for a carcass of this grade.

(2) A Hereford heifer, beginning at 6 months of age and at a weight of 319 lb., was fed for a period of 10 years a ration low in carotene and vitamin A but otherwise adequate, for the purpose of studying the vitamin A requirements for reproduction. At first sufficient alfalfa leaf meal to furnish 30 μg of carotene per kilogram of body weight was fed daily, but at the end of 4 years the carotene was increased to 60 μg. The ration consisted of the following ingredients: barley 40%, oats 30%, bran 10%, dried beet pulp 10%, linseed oil meal 4%, soybean meal 4%, iodized salt 1%, and limestone 1%. The ration contained 14.6% protein, 9.1% fiber, 3.2% fat, 0.54% Ca, and 0.47%P. Oat straw was fed as roughage. During a 10-year period the cow gave birth to a number of calves. At the time of slaughter she weighed 1,460 lb. and was in excellent condition. The chilled carcass weighed 940 lb. and was graded "commercial." It was very fat and there was an unusual distribution of intramuscular fat but no evidence of fatty degeneration.

The carcass of each animal was divided into the usual wholesale cuts and the following were selected for experimental purposes: round (rump and shank off), entire loin, rib, chuck, neck, and combined fore and hind shanks. The lean meat from each cut was cut into small pieces, frozen at 0°F., ground and dehydrated at 120°F. Considerable difficulty was experienced in the dehydration of the lean meat of certain cuts from the Hereford cow because of the unusual deposits of intramuscular fat. The dehydrated beef was thoroughly extracted with ethyl ether, ground fine and stored in friction-top tin cans at approximately 0°F.

Dried skim milk of a well-known brand, packed in sealed tin cans, was also tested.

Beef sample 4533, used in an experiment with gelatin, was a mixture of equal proportions of dehydrated chuck, round and loin from the Hereford cow.

Diets fed

The dehydrated beef from each cut from each carcass and dried skim milk were incorporated in diets in such proportions as to supply 1.2, 1.6 and 2% of nitrogen corresponding to 7.5, 10 and 12.5% protein. In addition, diets containing 1.2% nitrogen from beef were supplemented with 0.25% L-cystine, since Hoagland et al. ('48) had previously found beef protein to be deficient in this amino acid. Diets containing 1.6% nitrogen were also prepared in which 10, 15 or 20% of the total nitrogen was supplied as gelatin and the remainder as dehydrated beef.

The following quantities of B vitamins were added to 100 gm of each diet: thiamine hydrochloride 0.3 mg, riboflavin 0.3 mg, pyridoxine hydrochloride 0.6 mg, calcium pantothenate 1.5 mg, and choline chloride 80 mg.

The fat-soluble vitamins were dissolved in lard and added in such quantity that 1 gm of the diet would contain 5 I. U. of vitamin A, one unit of vitamin D and 20 μg of alpha-tocopherol. Sufficient kettle-rendered lard was added to make 10% fat in the diet. Salt mixture (Hoagland and Snider, '40) amounting to 4% and sufficient dextrin to make up 100% completed the diet. Each diet was made up in the quantity of 1 kg at one time and was stored in one-quart glass jars in a refrigerator at 40°F. during the course of the experiments.

The feeding tests were conducted in the manner previously described by the present writers ('47).

RESULTS

Experiment 1

The average results of the feeding tests with groups of 8 young male albino rats to determine the relative growth-promoting values of the protein in 6 cuts of beef from the carcass of a Shorthorn steer are shown in table 1. The gain in weight per gram of nitrogen consumed was selected as the most reliable basis for comparisons in this and the following experiments.

TABLE 1

Nutritive value of the protein in different cuts of beef from the carcass of a Shorthorn steer in 30-day tests with groups of 8 male albino rats

DESCRIPTION OF DIETS		PROTEIN CONTENT	NITROGEN CONTENT	GAIN IN WEIGHT	FEED CONSUMED	FEED CONSUMED PER GRAM GAIN IN WEIGHT	GAIN IN WT. PER GRAM OF NITROGEN
no.	Cut of beef	%	%	gm	gm	gm	gm
		Experiment 1					
1	Shank	7.5	1.2	85	323	3.80	21.9
2	Round	7.5	1.2	75	282	3.76	22.1
3	Loin	7.5	1.2	76	290	3.82	21.7
4	Neck	7.5	1.2	78	300	3.85	21.6
5	Rib	7.5	1.2	72	284	3.94	20.9
6	Chuck	7.5	1.2	81	309	3.81	21.7
	Average	7.5	1.2	78	298	3.83	21.7
7	Shank	10.0	1.6	118	344	2.92	21.4
8 [1]	Round	10.0	1.6	115	332	2.88	21.7
9	Loin	10.0	1.6	125	356	2.85	21.8
10	Neck	10.0	1.6	121	350	2.89	21.6
11	Rib	10.0	1.6	120	348	2.90	21.5
12	Chuck	10.0	1.6	126	360	2.86	21.9
	Average	10.0	1.6	121	348	2.88	21.7
13	Shank	12.5	2.0	151	374	2.48	20.1
14	Round	12.5	2.0	157	383	2.44	20.5
15	Loin	12.5	2.0	148	374	2.53	19.8
16	Neck	12.5	2.0	151	386	2.56	19.5
17	Rib	12.5	2.0	142	368	2.59	19.1
18	Chuck	12.5	2.0	158	390	2.47	20.3
	Average	12.5	2.0	151	379	2.51	19.9
19	Shank + 0.25% cystine	7.5	1.23	109	340	3.12	26.0
20	Round + 0.25% cystine	7.5	1.23	114	340	2.73	27.3
21	Loin + 0.25% cystine	7.5	1.23	117	345	2.95	27.5
22	Neck + 0.25% cystine	7.5	1.23	113	351	3.11	26.2
23	Chuck + 0.25% cystine	7.5	1.23	121	366	3.02	26.8
	Average	7.5	1.23	115	348	2.99	26.8

[1] Seven rats.

A statistical analysis of the experimental data was made by means of Fisher's "t" values for the significance of the difference between the means of the gain in weight per gram of nitrogen consumed. The following probability values were obtained:

(1) 1.2% nitrogen, diet 5 vs. diets 1, 2, 3, 4, and 6—not significant
(2) 1.6% nitrogen, diet 7 vs. diets 8, 9, 10, 11, and 12—not significant
(3) 2.0% nitrogen, diet 17 vs. diets 13, 14, and 18—significant (<0.02, 0.001, 0.01); diet 16 vs. diets 13, 14, and 18—significant (<0.05, 0.001, and 0.02)
(4) 1.2% nitrogen + 0.25% cystine, diet 19 vs. diets 20, 21, 22, and 23—not significant
(5) 1.2% nitrogen vs. 1.2% nitrogen + 0.25% cystine, diet 1 vs. 19, 2 vs. 20, 3 vs. 21, 4 vs. 22, and 6 vs. 23—all significant (<0.001)

The results of the tests with diets 1 to 6 (table 1) indicate clearly that there were no significant differences between the growth-promoting values for the protein in the different cuts of beef when the diets contained 1.2% nitrogen.

When the diets contained 1.6% nitrogen, the results of the tests with diets 7 to 12 (table 1) likewise show no significant differences between the growth-promoting values for the different cuts of beef.

When the diets contained 2.0% nitrogen, the growth-promoting values for the protein in the neck and rib (diets 16 and 17) were significantly lower than the values for the shank, round and chuck (diets 13, 14 and 18). The average growth-promoting value of the three less expensive cuts—the shank, neck and chuck—was 20.0, as compared with 19.8 for the three more expensive cuts, the round, loin and rib.

The addition of 0.25% cystine to diets 19 to 23, containing 1.2% protein nitrogen, caused a marked increase in the rate of growth and efficiency of protein utilization as compared with diets 1 to 6, which did not contain added cystine. There were no significant differences between the growth-promoting values of the protein in any of the cuts of beef when supplemented with cystine. The rib cut of beef was not tested because of an inadequate supply of meat.

The results of the experiments with the 6 cuts of beef from the Shorthorn steer show that there were no significant differences between the growth-promoting values of the protein in the different cuts when the diets contained 1.2 or 1.6% nitrogen, or 1.2% protein nitrogen + 0.25% cystine. Only when the diets contained 2.0% nitrogen was a significant difference found between the protein values for the rib and neck and three other cuts. The reason for these differences is not apparent. On the whole, the results of this experiment indicate that the protein in the less expensive cuts, the shank, neck and chuck, was of as high nutritive value as the protein in the more expensive cuts, the round, loin and rib.

It is noteworthy that the average growth-promoting value of the protein in all cuts of beef was identical at the 1.2 and 1.6% levels of nitrogen intake, and the value at the 2.0% nitrogen level was only slightly lower. On the other hand, there was a marked increase in the rate of growth of the rats as the percentage of protein was increased.

Experiment 2

This experiment was a duplicate of experiment 1 except that the cuts of beef were obtained from the carcass of a Hereford cow that had been fed a ration low in carotene for a long period of time. The results are shown in table 2.

A statistical analysis was made of the experimental data by the procedure followed in the preceding experiment, with the following results:

(1) 1.2% nitrogen, diet 2A vs. 3A, 4A, 5A, and 6A—significant (<0.05, 0.03, 0.05, 0.01). 2A vs. 1A, not significant (<0.1)
(2) 1.6% nitrogen, differences not significant
(3) 2.0% nitrogen, diet 17A vs. 18A—significant (<0.02); other differences not significant
(4) 1.2% nitrogen + 0.25% cystine, diet 23A vs. 19A, 20A, 21A, and 24A—significant (<0.05, 0.05, 0.01, 0.01); other differences not significant
(5) 1.2% nitrogen vs. 1.2% nitrogen + 0.25% cystine, diets 1A vs. 19A, 2A vs. 20A, 3A vs. 21A, 4A vs. 22A, 5A vs. 23A, 6A vs. 24A—all significant (<0.001)

TABLE 2

Nutritive value of the protein in different cuts of beef from the carcass of a Hereford cow in 30-day tests with groups of 8 male albino rats

DESCRIPTION OF DIETS		PROTEIN CONTENT	NITROGEN CONTENT	GAIN IN WEIGHT	FEED CONSUMED	FEED CONSUMED PER GRAM GAIN IN WEIGHT	GAIN IN WT. PER GRAM OF NITROGEN
no.	Cut of beef						
		%	%	gm	gm	gm	gm
		Experiment 2					
1A	Shank	7.5	1.2	67	263	3.93	21.1
2A	Round	7.5	1.2	74	276	3.73	22.2
3A	Loin	7.5	1.2	69	278	4.03	20.4
4A	Neck	7.5	1.2	67	272	4.06	20.4
5A	Rib	7.5	1.2	71	279	3.93	21.1
6A	Chuck	7.5	1.2	70	289	4.13	20.1
	Average	7.5	1.2	70	276	3.97	20.9
7A	Shank	10.0	1.6	97	295	3.04	20.6
8A	Round	10.0	1.6	100	315	3.15	19.9
9A	Loin	10.0	1.6	100	316	3.16	19.7
10A	Neck	10.0	1.6	104	307	2.95	21.1
11A	Rib	10.0	1.6	100	302	3.02	20.6
12A	Chuck	10.0	1.6	98	316	3.22	19.3
	Average	10.0	1.6	100	309	3.09	20.2
13A	Shank	12.5	2.0	131	342	2.61	19.2
14A	Round	12.5	2.0	143	355	2.48	20.1
15A	Loin	12.5	2.0	141	357	2.53	19.7
16A	Neck	12.5	2.0	135	351	2.60	19.3
17A	Rib	12.5	2.0	125	329	2.63	18.9
18A	Chuck	12.5	2.0	139	347	2.50	20.0
	Average	12.5	2.0	136	347	2.56	19.5
19A	Shank + 0.25% cystine	7.5	1.23	109	331	3.04	26.7
20A	Round + 0.25% cystine	7.5	1.23	104	316	3.04	26.6
21A	Loin + 0.25% cystine	7.5	1.23	102	322	3.16	25.8
22A	Neck + 0.25% cystine	7.5	1.23	114	345	3.03	26.8
23A	Rib + 0.25% cystine	7.5	1.23	119	344	2.89	28.0
24A	Chuck + 0.25% cystine	7.5	1.23	88	284	3.23	25.2
	Average	7.5	1.23	106	324	3.07	26.5

The results of the experiment with the diets containing 1.2% nitrogen and using the different cuts from the carcass of the Hereford cow suggest that the protein in the round was of higher nutritive value than that in the other cuts, except the shank, but there were no other significant differences. The fact that the protein in the round was not superior to that in the other cuts at the higher levels of intake indicates that the apparent superiority of the round at the 1.2% level of nitrogen intake was fortuitous.

When the diets contained 1.6% nitrogen there were no significant differences between the growth-promoting values of the protein in the different cuts of beef.

The results of the tests with the diets containing 2% nitrogen indicate that the protein in the chuck (diet 18A) was of significantly higher nutritive value than that in the rib (diet 17A). Other differences were not significant.

When the diets contained 1.2% nitrogen + 0.25% cystine there was a marked increase in the rate of growth and in the efficiency of utilization of the protein in each cut of beef as compared with the diets containing 1.2% nitrogen but no added cystine. The protein in diet 23A (rib + cystine) had an unusually high growth-promoting value, significantly higher than that for any other diet except 22A (neck + cystine).

In this experiment there were only relatively small differences between the average growth-promoting values for all cuts at the 7.5, 10, and 12.5% levels of protein intake. The average growth value for the protein at each level of intake was slightly higher for the cuts from the Shorthorn steer (table 1) than for those from the Hereford cow (table 2) but a statistical analysis of the experimental data indicates that the differences were not significant.

Experiment 3

This experiment was conducted to determine (1) the growth-promoting value of the protein in dried skim milk at the 1.2, 1.6, and 2.0% levels of nitrogen intake, and (2)

the effects of the addition to beef of different proportions of gelatin on the nutritive value of the protein at the 1.6% level of nitrogen intake. In order to simplify comparisons between diets in this and previous experiments, the factor 6.25 was used to convert nitrogen to protein. The results are shown in table 3.

TABLE 3

Nutritive value of the protein in dried skim milk and in mixtures of dried raw beef and gelatin in 30-day tests with groups of 8 male albino rats

DESCRIPTION OF DIETS		PROTEIN[1] CONTENT	NITROGEN CONTENT	GAIN IN WEIGHT	FEED CONSUMED	FEED CONSUMED PER GRAM GAIN IN WEIGHT	GAIN IN WT. PER GRAM OF NITROGEN
no.	Source of protein	%	%	*gm*	*gm*	*gm*	*gm*
		Experiment 3					
1B	Dried skim milk	7.5	1.2	88	328	3.73	22.2
2B	Dried skim milk	10.0	1.6	124	375	3.02	20.6
3B	Dried skim milk	12.5	2.0	132	362	2.74	18.2
4B	Dried beef 4533	10.0	1.6	124	367	2.96	21.0
5B	Dried beef 4533 + 10% gelatin nitrogen	10.0	1.6	107	348	3.25	19.2
6B	Dried beef 4533 + 15% gelatin nitrogen	10.0	1.6	109	352	3.23	19.3
7B	Dried beef 4533 + 20% gelatin nitrogen	10.0	1.6	97	337	3.47	17.9

[1] Protein content = N × 6.25.

A statistical analysis was made of the experimental data with the following results:

(1) Diet 1B vs. 2B and 3B—significant (<0.01, 0.001)
(2) Diet 2B vs. 3B—significant (<0.02)
(3) Diet 1B vs. average of diets 1 to 6—not significant
(4) Diet 1B vs. average of diets 1A to 6A—significant (<0.02)
(5) Diet 2B vs. average of diets 7 to 12—significant (<0.01)
(6) Diet 2B vs. average of diets 7A to 12A—not significant
(7) Diet 3B vs. average of diets 13 to 18—significant (<0.001)
(8) Diet 3B vs. average of diets 13A to 18A—significant (0.001)
(9) Diet 4B vs. 5B, 6B, 7B—significant (<0.001, 0.01, 0.001)
(10) Diet 5B vs. 7B—significant (<0.01)
(11) Diet 6B vs. 7B—significant (<0.05)

The results of the experiments with dried skim milk show an increase in the rate of growth of the rats but a decrease in the efficiency of utilization of the protein as the percentage of nitrogen in the diets was increased from 1.2 to 1.6 or 2.0%. A comparison of the growth-promoting value of milk protein (gain per gram of nitrogen consumed) at each level of intake (table 3) with the average values for all cuts of beef at the same level of intake (tables 1 and 2) yields the following results: (1) At the 1.2% nitrogen level, the protein in dried skim milk had approximately the same growth value as all cuts of beef from the Shorthorn steer (table 1), but one significantly higher than the average value for all cuts from the Hereford cow (table 2); (2) At the 1.6% nitrogen level the average value for all cuts from the Shorthorn steer (table 1) was significantly higher than the value for milk, but the average value of the cuts from the Hereford cow (table 2) was approximately the same as that for milk; (3) At the 2.0% nitrogen level, the average value for all the cuts from each carcass of beef was significantly higher than the value for milk.

The admixture of different proportions of gelatin with dehydrated raw beef materially lowered the growth-promoting values of the mixtures as compared with those of beef alone. When nitrogen constituted 1.6% of the diet, the replacement of 10, 15 or 20% of the beef nitrogen by gelatin nitrogen resulted in a marked decrease in the rate of growth and in the efficiency of protein utilization. The fact that the protein in the less expensive cuts of beef, such as the neck, shank and chuck, did not differ consistently in growth-promoting value from the protein in the more expensive cuts such as the round, loin and rib, suggests that the difference in collagen content of the two groups of cuts is not sufficient materially to affect the nutritive value of the total protein in the meat.

SUMMARY

The relative nutritive or growth-promoting values of the protein in 6 different cuts of beef from each of two car-

casses, one of a Shorthorn steer graded "high good" and the other a Hereford cow graded "commercial," were determined by feeding tests with young male albino rats. The following cuts of beef were tested: round, loin, rib, chuck, neck, and combined fore and hind shanks.

When protein constituted 7.5% of the diets, there were no significant differences between the nutritive values of the protein in the different cuts from the Shorthorn steer. In tests with the cuts from the Hereford cow, the protein in the round had a higher value than that in the other cuts except the shanks.

When protein constituted 10% of the diets, there were no significant differences between the nutritive values of the protein in the different cuts of beef, either from the Shorthorn steer or from the Hereford cow.

When the diets contained 12.5% protein, the growth-promoting values of the protein in the rib and neck from the Shorthorn steer were slightly lower than the values for the shank, round or chuck. The protein in the rib from the Hereford cow had a slightly lower value than the protein in the chuck.

The average growth-promoting value of the protein at each level of intake was slightly higher for all the cuts from the Shorthorn steer than for those from the Hereford cow, but the differences were not statistically significant.

When diets containing 7.5% protein were supplemented with 0.25% cystine, there was a marked increase in the rate of growth and in the efficiency of utilization of the protein in each cut of beef from each animal. There were no significant differences between the nutritive values of the protein in the different cuts from the Shorthorn steer. The protein in the rib from the Hereford cow had a significantly higher value than that in any other cut, except the neck.

The average nutritive value of the protein in the cuts of beef from the Shorthorn steer, at the 7.5% level of protein intake, was approximately the same as the value for dried skim milk. When the diets contained 10.0 or 12.5% protein,

the beef protein was of higher nutritive value than the milk protein.

The average nutritive value of the protein in the cuts from the Hereford cow was lower than the value for milk at the 7.5% protein level, the same at the 10% level, but higher at the 12.5% level.

The admixture of gelatin with beef, in the proportions of 10, 15, or 20% of the total nitrogen in the diets, definitely lowered the nutritive value of the total protein when the diets contained 1.6% nitrogen.

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PROCEEDINGS OF THE THIRTEENTH ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

THE STATLER HOTEL AND THE MASONIC TEMPLE
DETROIT, MICHIGAN, APRIL 18–22, 1949

COUNCIL MEETINGS

Council meetings were held in the Statler on Sunday and Monday, April 17 and 18. Formal actions of the Council are reported in the minutes of the business meetings.

SCIENTIFIC SESSIONS

The scientific program consisted of 8 half-day sessions of papers grouped according to subject matter and a symposium on "Nutrition in Preventive Medicine" held on the evening of April 21. Ninety-four papers were presented at the scientific sessions and 4 papers were read by title. The Institute also participated in the Joint Session of the Federation on the evening of April 19.

BUSINESS SESSIONS

Two business meetings were held: one at 4:30 P.M., Tuesday, April 19, and one at 4:30 P.M., Thursday, April 21. President E. M. Nelson presided at the first meeting and Vice-President C. G. King was in charge of the second meeting. Items of business received attention as follows:

Tuesday, April 19, 4:30 P.M. The meeting was called to order by President E. M. Nelson. The President appointed Dr. H. R. Bird and Dr. A. Arnold to serve as a Tellers' Committee and ballots on the election of officers were transmitted to this Committee by the Secretary.

President Nelson referred to the Proceedings of last year's meeting, as pubished in the June, 1948, issue of THE JOURNAL OF NUTRITION, and a call was made for corrections or additions. No objections being noted, the published transactions of last year's meeting were declared approved.

The President reported that since the last meeting the Special Committee charged with the responsibility of setting up the rules and regulations for the award made available by the Nutrition Foundation, Inc., had recommended that this award be called the "Osborne and Mendel Award." This recommendation had been approved by the Council.

The report of the Treasurer, N. R. Ellis, was submitted. The Auditing Committee (Dr. Elsa Orent-Keiles and Dr. Esther L. Batchelder) presented a report stating that the books of the Treasurer were in order. The Treasurer's report was adopted.

The Secretary reported that the Executive Committee of the Federation had taken the following actions:

Decided that the next annual meeting would be held at Atlantic City, New Jersey, during the week beginning April 9.

Recommended that the Federation assessment be set at three dollars per member.

Recommended that the secretaries of the constituent societies serve as the Editorial Board for the Federation Proceedings, replacing the Control Board which has been functioning in this capacity.

Dr. H. J. Deuel, Jr., reported that Dr. George R. Cowgill had been elected by the Editorial Board of THE JOURNAL OF NUTRITION to serve as Editor for the coming 5-year period, and that Dr. Cowgill had accepted. Dr. Deuel read the following motion which had been unanimously adopted by the Editorial Board:

"It was moved that the Editor be cordially thanked for his services during the past 10 years, that he be re-elected for the ensuing 5 years, and that he be urgently requested to accept the action of the Board."

The annual report of the Editor of THE JOURNAL OF NUTRITION was submitted by Dr. G. R. Cowgill. The report follows:

Two volumes (Nos. 35 and 36) were published during 1948; they contained 64 and 67 articles, respectively, which was an increase of 10 papers over the number published in 1947. The number of pages per article proved to be almost identical with that for the previous year.

During 1948, because of rising publication costs, Wistar Institute found it necessary to review every aspect of its printing shop operations. Following its study, the subscription price for most of the Wistar publications was increased and certain other actions taken. With respect to THE JOURNAL OF NUTRITION (a) the subscription rate to non-society members was raised from $5.00 to $7.00 per volume beginning in January, 1949; (b) the number of volumes to be published annually was increased from two to three; (c) the number of pages per volume was reduced from 720 to 600, the net effect of which is the publication of at least 1800 instead of 1440 pages per year; and finally, (d) the Editor was allowed additional pages not to exceed 200 annually as he might need them in order to secure the most prompt publication of acceptable material. The Editor proposed to Wistar Institute — and it was accepted — that there be no change in the subscription rate for members of the American Institute of Nutrition, who are required to subscribe as a condition of membership. Inasmuch as (a) members of the society had already paid through annual dues for two volumes of the Journal and thus were entitled to issues up to and including that for April, 1949, and (b) new dues would have to be voted at the next annual meeting scheduled for Detroit in April, 1949, the Editor further proposed to Wistar Institute — and it was accepted — that any change in the annual cost of the Journal to society members not be effective until after the next annual meeting, at which annual dues would be discussed and then voted. If the new arrangement is adopted, the part of the annual dues

covering subscription to the JOURNAL (for three volumes) will be $7.50.

A cumulative index covering a 10-year period (1938–1948) and 21 volumes (16–36 inclusive) is being prepared. It should be ready during the summer of 1949.

A motion was passed setting the total dues of members at $11.50 for the next year. Of this amount, $1.00 is allocated to the Institute of Nutrition, $3.00 to the Federation and $7.50 for subscription to THE JOURNAL OF NUTRITION.

Dr. G. R. Cowgill discussed in further detail the preparation of the 10-year cumulative index. He presented the request of the Wistar Institute of Anatomy and Biology that the American Institute of Nutrition assume financial responsibility for publishing the index to the extent of the cost of one volume per member. Dr. J. R. Murlin moved that, if a deficit occurs in publishing the index, the Institute of Nutrition would assume responsibility equal to the cost of one copy per member, less a credit for the number of copies that have been purchased by members. The motion was passed.

A motion was passed authorizing the Secretary to prepare and distribute with the ballots for election of officers a printed directory of members with their affiliations.

The report of the Committee on Pathological Registries, consisting of Dr. R. E. Johnson, Chairman, Dr. O. A. Bessey and Dr. W. H. Sebrell, on the desirability and feasibility of sponsoring a Registry of Nutritional Pathology as a new unit of the American Registry of Pathology was presented by Dr. Johnson. The Committee recommended that such a registry be sponsored by the American Institute of Nutrition and submitted an organization plan by which this registry would be set up. The report was adopted.

President Nelson requested authority to appoint a commitee to study present procedures used in selecting recipients of awards and in conferring these awards and to make recommendations at the next annual meeting that will lead to improved procedures. The request was approved.

The Tellers' Committee reported the election of the following officers for the year beginning July 1, 1949:

President
CHARLES G. KING
Vice President
WENDELL H. GRIFFITH
Councillor
ARTHUR H. SMITH
Associate Editors
WILLIAM J. DARBY
DAVID M. HEGSTED
PEARL P. SWANSON

The meeting was adjourned at 5:20 P.M.

Thursday, April 21, 4:30 P.M. The second business meeting was called to order by Vice President C. G. King. The Tellers' Committee reported the results of the voting upon suggestions for the nominating committee. The names of the 10 members receiving the highest number of votes were submitted for the guidance of the President.

The Secretary presented the report of the Committee on Nomenclature (C. A. Elvehjem, Chairman; E. M. Nelson, A. D. Welsh, H. J. Almquist). The report was adopted. This report is as follows:

> "It is recommended that the name folic acid and the synonym folacin be adopted for the compound which has been identified as N-[4-{[(2-amino-4-hydroxy-6-pteridyl) methyl] amino} benzoyl] glutamic acid and which has previously been designated by several names including pteroylglutamic acid, vitamin B_c and *L. casei* factor.
>
> It is recommended that the name pyridoxal be adopted for the compound which has been identified as 2-methyl-3-hydroxy-4-formyl-5-hydroxy-methylpyridine.
>
> It is recommended that the name pyridoxamine be adopted for the compound which has been identified as 2-methyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine.
>
> It is further recommended that the term vitamin B_6 be be used as a group name to include pyridoxal, pyridoxamine and pyridoxine, and that these specific names be used only where the corresponding chemical compound is meant. (This recommendation specifically recognizes that the terms 'vitamin B_6' and 'pyridoxine' should no longer be used synonymously.)"

The Secretary presented a resolution submitted by the Executive Committee of the Federation concerning policies used in selecting and developing the Medical Service Corps, Medical Allied Sciences Section, U.S. Army. A motion was passed adopting the resolution and recommending that it be transmitted to Surgeon General R. W. Bliss. The resolution is as follows:

"The Executive Committee of the Federation of American Societies for Experimental Biology recommends to the respective member societies that they transmit to the office of the Surgeon General, United States Army, attention, Major General R. W. Bliss, a resolution of the following nature:

> WHEREAS, the members of our organization are greatly interested in the development of policies that will lead to maximum military effectiveness of scientific personnel,
>
> Be it resolved that we respectfully urge further and continued consideration of the recommendations formulated at the Medical Service Corps Conference, Medical Allied Sciences Section, May 27 and 28, 1948; and
>
> Be it further resolved that in order to keep our members informed concerning their opportunities and responsibilities, similar conferences should be scheduled to review progress and to discuss the related problems of mutual interest."

The report of the Council on the election of new members was adopted.

The following persons were elected to membership in the American Institute of Nutrition:

James B. Allison
A. E. Axelrod
Elizabeth C. Callison
Dena C. Cederquist
Charles S. Davidson
Paul F. Fenton
Gottfried S. Fraenkel
Charles R. Grau
Max K. Horwitt
H. C. Hou
Carl J. Koehn
Frank H. Kratzer
Willard A. Krehl
Henry A. Lardy
Frieda L. Meyer
Walther H. Ott
Leo T. Samuels
Nevin S. Scrimshaw
William C. Sherman
Mary Speirs
Carleton R. Treadwell
Le Roy Voris

Discussion of the procedures by which awards are made brought out the points that a re-examination of the present procedures is in order and that efforts should be made to obtain greater stimulation and more publicity in regard to the awards made through the Institute of Nutrition.

There was considerable discussion concerning the desirability of presenting a better scientific program. Dr. E. W. McHenry presented a motion to have the President appoint a committee of 5 members to study present procedures regarding the program of the annual meeting and to make recommendations to the Program Committee and to the Institute at its next annual meeting of ways by which the scientific program may be improved. The motion was passed.

The meeting was adjourned at 5:20 P.M.

DINNER AND PRESENTATION OF AWARDS

The Institute held its annual dinner on Wednesday evening, April 20, 1949, in the Book-Cadillac Hotel.

Very stimulating addresses were made by President E. M. Nelson, Dr. W. C. Russell and Dr. C. G. King. Dr. Nelson spoke upon his work as a delegate to a meeting in London, June 10 and 11, 1948, of the Provisional Committee to consider the formation of an International Union of Nutrition Societies. At this conference it was agreed that the name of the proposed organization should be the "International Union of Nutrition Sciences." A tentative constitution and by-laws for the proposed union was prepared and adopted. Application has been made to the International Congress of Scientific Unions for the establishment of an International Union of Nutrition Sciences and this application will be acted upon in September, 1949. Dr. Russell spoke upon the activities of the National Research Council, with special reference to the work of the Food and Nutrition Board. Dr. King discussed the operation of the Medical Allied Services Section of the Medical Corps of the Army, particularly as it relates to the development of scientific information and the opportunities for a scientific career in the Army.

The Mead Johnson Company Award was divided equally between two recipients, Dr. Karl A. Folkers and Dr. Mary S. Shorb. The award was granted to Dr. Folkers for "isolating in crystalline form a new chemical substance, designated as vitamin B_{12}, that is active in the treatment of pernicious anemia and of importance in other phases of nutrition," and to Dr. Shorb for "pointing out the possible connection between the anti-anemia factor and a growth factor for bacteria and for developing a bacteriological assay procedure that materially assisted in the isolation of the compound designated as vitamin B_{12}."

The Borden Award in Nutrition was presented to Dr. Harry J. Deuel, Jr., for "research emphasizing the nutritive importance of components of dairy products."

The Osborne and Mendel Award was presented to Dr. William C. Rose for "his contributions to our knowledge of nitrogen metabolism and the amino acid requirements of experimental animals and man."

COMMITTEES FOR 1949–1950

President E. M. Nelson appointed the following committees for the year beginning July 1, 1949:

Nominating Committee

Icie Macy Hoobler, *Chairman*
E. W. Crampton
H. J. Deuel, Jr.
L. A. Maynard
F. J. Stare

Committee on Rules for Awards

W. C. Russell, *Chairman*
H. E. Carter
R. R. Sealock
C. D. Tolle
J. Waddell

Committee on Program Policy

W. H. GRIFFITH, *Chairman*
G. R. COWGILL
F. S. DAFT
R. M. LEVERTON
E. W. MCHENRY

Committee on Registry of Nutritional Pathology

R. E. JOHNSON, *Chairman*
O. A. BESSEY
P. L. PHILLIPS
W. H. SEBRELL

Institute Representatives on Joint Committee on Nomenclature

H. J. ALMQUIST
C. A. ELVEHJEM

Respectfully submitted,
JOSEPH H. ROE, *Secretary*
American Institute of Nutrition